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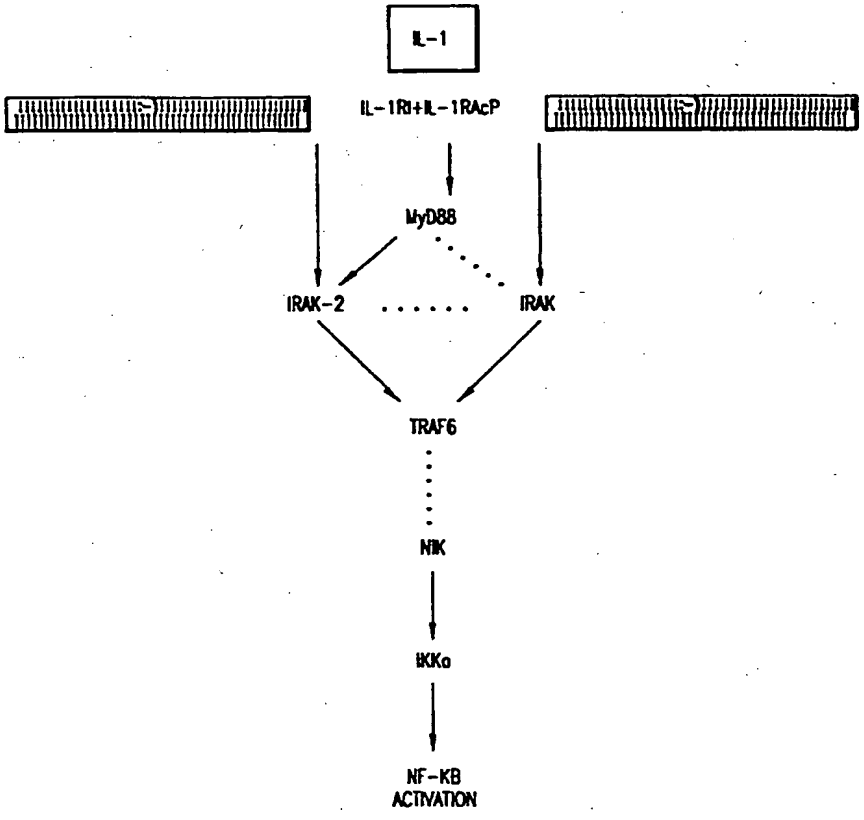
(57) Abstract

The present invention relates to a novel IRAK–2 protein which is a member of the IL–1 signaling pathway. In particular, isolated nucleic acid molecules are provided encoding the human IRAK–2 protein. IRAK–2 polypeptides are also provided as are vectors, host cells and recombinant methods for producing the same. Also provided are diagnostic methods for detecting IRAK–2 related disorders and therapeutic methods for treating IRAK–2 related disorders.



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HUMAN IRAK-2, A HUMAN INTERLEUKIN-1 RECEPTOR ASSOCIATED KINASE-2

Field of the Invention

The present invention relates to a novel interleukin-1 receptor signaling protein. More specifically, isolated nucleic acid molecules are provided encoding a human interleukin-1 receptor associated kinase-2 (IRAK-2). IRAK-2 polypeptides are also provided, as are vectors, host cells and recombinant methods for producing the same.

Background of the Invention

Interleukin-1 (IL-1). Interleukin-1 (IL-1α and IL-1β) is a "multifunctional" cytokine that affects nearly every cell type, and often in concert with other cytokines or small mediator molecules. (Dinarello, C.A., *Blood 87*:2095-2147 (March 15, 1996).) There are three members of the IL-1 gene family: IL-1α, IL-1β, and IL-1 receptor antagonist (IL-1Ra). IL-1α and IL-1β are agonists and IL-1Ra is a specific receptor antagonist. IL-1α and β are synthesized as precursors without leader sequences. The molecular weight of each precursor is 31 kD. Processing of IL-1α or IL-1β to "mature" forms of 17 kD requires specific cellular proteases. In contrast, IL-1Ra evolved with a signal peptide and is readily transported out of the cells and termed secreted IL-1Ra (sIL-1Ra).

IL-1 Receptor and Ligands. The receptors and ligands of the IL-1 pathway have been well defined (for review, see Dinarello, C.A., *FASEB J. 8*:1314-1325 (1994); Sims, J.E. *et al.*, *Interleukin-1 signal transduction: Advances in Cell and Molecular Biology of Membranes and Organelles*, Vol. 3, JAI Press, Inc., Greenwich, CT (1994), pp. 197-222). Three ligands, IL-1α, IL-1β, and IL-1 receptor antagonist (IL-1Ra) bind three forms of IL-1 receptor, an 80-kDa type I IL-1 receptor (IL-1RI) (Sims, J.E. *et al.*, *Science 241*:585-589 (1988)), a 68-kDa type II IL-1 receptor (IL-1RII) (McMahan, C.J. *et al.*, *EMBO J. 10*:2821-2832 (1991)), and a soluble form of the type II IL-1R (sIL-1RII) (Colotta, F. *et al.*, *Science 261*:472-475 (1993)).

IL-1 production in various disease states. Increased IL-1 production has been reported in patients with various viral, bacterial, fungal, and parasitic infections; intravascular coagulation; high-dose IL-2 therapy; solid tumors; leukemias; Alzheimer's disease; HIV-1 infection; autoimmune disorders; trauma (surgery); hemodialysis; ischemic diseases (myocardial infarction); noninfectious hepatitis; asthma; UV radiation; closed head injury; pancreatitis; periodontitis; graft-versus-host disease; transplant rejection; and in healthy subjects after strenuous exercise. There is an association of increased IL-1β production in patients with Alzheimer's disease and a possible role for IL-1 in the release of the amyloid precursor protein (Vasilakos, J.P., *et al.*, *FEBS Lett. 354*:289 (1994)). However, in most conditions, IL-1 is not the only cytokine exhibiting increased production and hence the specificity of the IL-1 findings as related to the pathogenesis of any particular disease is lacking. In various disease states, IL-1β, but not IL-1α, is detected in the circulation.

IL-1 in Therapy. Although IL-1 has been found to exhibit many important biological activities, it is also found to be toxic at doses that are close to therapeutic dosages (Dinarello, C.A., *Blood 87*:2095-2147 (March 15, 1996)). In general, the acute toxicities of either isoform of IL-1 were greater after intravenous compared with subcutaneous injection. Subcutaneous injection was associated with significant local pain, erythema, and swelling (Kitamura, T., & Takaku, F., *Exp. Med.* 7:170 (1989); Laughlin, M.J., *Ann. Hematol. 67*:267 (1993)). Patients receiving intravenous IL-1 at doses of 100 ng/kg or greater experienced significant hypotension. In patients receiving IL-1β from 4 to 32 ng/kg subcutaneously, there was only one episode of hypotension at the highest dose level (Laughlin, M.J., *Ann. Hematol. 67*:267 (1993)).

Contrary to IL-1-associated myelostimulation in patients with normal marrow reserves, patients with aplastic anemia treated with 5 daily doses of IL-1α (30 to 100 ng/kg) had no increases in peripheral blood counts or bone marrow cellularity (Walsh, C.E., *et al.*, *Br. J. Haematol 80*:106 (1992)). IL-1 has been

administered to patients undergoing various regiments of chemotherapy to reduce the nadir of neutropenia and thrombocytopenia.

Daily treatment with 40 ng/kg IL-1α from day 0 to day 13 of autologous bone marrow or stem cells resulted in an earlier recovery of neutropenia (median, 12 days; $P < .001$) (Weisdorf, D., *et al.*, *Blood 84*:2044 (1994)). After 14 days of treatment, the bone marrow was significantly enriched with committed myeloid progenitor cells. Similar results were reported in patients with AML receiving 50 ng/kg/d of IL-1β for 5 days starting at the time of transplantation with purged or nonpurged bone marrow (Nemunaitis, J., *et al.*, *Blood 83*:3473 (1994)). Injecting humans with low doses of either IL-1α or IL-1β confirms the impressive pyrogenic and hypotension-inducing properties of the molecules.

IL-1 signaling mechanisms. After binding to interleukin-1 (IL-1), the IL-1 receptor type 1 (IL-1RI) associates with the IL-1R Accessory Protein (IL-1RAcP) and initiates a signaling cascade that results in the activation of NF-kB, (Greenfeder, S.A., *et al.*, *J. Biol. Chem. 270*:13757-65 (1995); Sims, J.E., *et al.*, *Science 241*:585-9 (1988); Korherr, C.,*et al.*, *Eur. J. Immunol. 27*:262-7 (1997); Wesche, H., *et al.*, *J. Biol. Chem. 272*:7727-31 (1997); Freshney, N.W., *et al.*, *Cell 78*:1039-49 (1994); and Martin, M., *et al.*, *Eur. J. Immunol. 24*:1566 (1994)). Significant similarity exists between the IL-1R signaling pathway in mammals and the Toll signaling pathway in *Drosophila*. Toll, which shares sequence homology with the cytoplasmic domain of the IL-1RAcP, induces Dorsal activation (a homologue of NF-kB) via the adapter protein Tube and the protein kinase Pelle, (Galindo, R.L., *et al.*, *Development 121*:2209-18 (1995); Norris, J.L. & Manley, J.L., *Genes Devel. 10*:862-72 (1996); Letsou, A., *et al.*, *EMBO 12*:3449-3458 (1993); and Grosshans, J., *et al.*, *Nature 372*:563-566 (1994)); significantly the recently identified IRAK (IL-1R Associated Kinase) is homologous to Pelle, (Cao, Z., *et al.*, *Science 271*:1128-31 (1996)). However, in mammalian cells, additional complexity is thought to exist based on the observation that multiple protein kinase activities coprecipitate with the IL-1RI (Singh, R., *et al.*, *J. Clin. Invest. 100*:419 (1997); and Eriksson, A., *et al.*,

Cytokine 7:649 (1995)). Furthermore, given that in *Drosophila* the adapter protein Tube interacts with and regulates Pelle's activity, it is likely that analogous adapter/regulatory molecules might participate in IL-1 signaling. There is a need in the art to characterize molecules involved in the IL-1 signaling pathway.

Nuclear factor kappa B (NF-kB). NF-kB is a member of a family of dimeric transcription factors made from monomers that have approximately 300 amino-acid Rel regions which bind to DNA, interact with each other, and bind the IkB inhibitors (for review, see Baeuerle and Baltimore, *Cell 87*:13-20 (1996)). Disregulation of NF-kB has been implicated in malignant transformation and hyperplasia (Gilmore *et al.*, *Oncogene 9*:2391-2398 (1996)). NF-kB plays an important role in the antiviral response as a virus-inducible transcriptional regulator of β-interferon, MHC class I, and inflammatory cytokine genes. NF-kB has also been shown to protect cells from pro-apoptotic stimuli (Beg *et al.*, *Nature 376*:167-170 (1995)).

Summary of the Invention

The present invention provides isolated nucleic acid molecules comprising a polynucleotide encoding the IRAK-2 polypeptide having the amino acid sequence shown in SEQ ID NO:2 or SEQ ID NO:4 or the amino acid sequence encoded by the cDNA clone deposited in a bacterial host as ATCC Deposit Number 209340 on October 7, 1997.

The present invention also relates to recombinant vectors, which include the isolated nucleic acid molecules of the present invention, and to host cells containing the recombinant vectors, as well as to methods of making such vectors and host cells and for using them for production of IRAK-2 polypeptides or peptides by recombinant techniques.

The invention further provides an isolated IRAK-2 polypeptide having an amino acid sequence encoded by a polynucleotide described herein.

The present invention also provides a screening method for identifying compounds capable of enhancing or inhibiting a cellular response induced by the IRAK-2, which involves contacting cells which express the IRAK-2 with the candidate compound, assaying a cellular response, and comparing the cellular response to a standard cellular response, the standard being assayed when contact is made in absence of the candidate compound; whereby, an increased cellular response over the standard indicates that the compound is an agonist and a decreased cellular response over the standard indicates that the compound is an antagonist.

The invention provides a diagnostic method useful during diagnosis of a IRAK-2 or IL-1 disorder.

An additional aspect of the invention is related to a method for treating an individual in need of an increased level of IRAK-2 activity in the body comprising administering to such an individual a composition comprising a therapeutically effective amount of an isolated IRAK-2 polypeptide of the invention or an agonist thereof.

A still further aspect of the invention is related to a method for treating an individual in need of a decreased level of IRAK-2 activity in the body comprising, administering to such an individual a composition comprising a therapeutically effective amount of an IRAK-2 antagonist.

Brief Description of the Figures

Figure 1 shows the nucleotide (SEQ ID NO:1) and deduced amino acid (SEQ ID NO:2) sequences of IRAK-2α. The protein has a deduced molecular weight of about 65 kDa.

Figure 2 shows the nucleotide (SEQ ID NO:3) and deduced amino acid (SEQ ID NO:4) sequences of IRAK-2β.

Figure 3 shows the regions of similarity between the amino acid sequences of the IRAK-2α (SEQ ID NO:2) and IRAK-2β (SEQ ID NO:4) proteins and

human IRAK (SEQ ID NO:5) and Pelle (SEQ ID NO:6). Alignment was performed with Custall software.

Figure 4. Figure 4A shows that ectopic expression of IRAK-2 but not the mutant version of IRAK-2 (1-96) activates NF-kB in 293 cells as measured by NF-kB reporter gene activity. Figure 4B shows that IRAK-2 (1-96) and IRAK-2 (97-590) inhibit IL-1Rs-induced NF-kB activity. Transfection with TRAF-2 (87-501) and NIK (KK429-430AA) expression vectors served as negative and positive controls, respectively. 0.1 μg of IL-1RI plus 0.1 μg of IL-1RAcP and 0.6 μg of putative inhibitory expression constructs were transfected. Data are expressed as percentage of relative IL-1Rs-induced NF-kB activity.

Figure 5 shows that IRAK-2 induced NF-kB activity is specifically abrogated by TRAF6 (289-522) but not TRAF2 (87-501). 293 cells were transfected with 0.2 μg of IRAK-2 and increasing amounts of TRAF constructs.

Figure 6. Figure 6A shows that ectopic expression of MyD88 in 293 cells results in the induction of NF-kB activity. A mutant version of MyD88 encoding a N-terminal region, MyD88 (1-152), was similarly capable of inducing NF-kB activity albeit to a lesser extent; in contrast a mutant version of MyD88 coding for amino acids 152 to the end, MyD88 (152-296) failed to induce any luciferase activity (not evident in graph). Figure 6B shows that MyD88-induced NF-kB activity was selectively inhibited by a dominant negative version of TRAF6, TRAF6 (298-522) but not TRAF2 (87-501). 0.1 µg of MyD88 and increasing amount of TRAF expression constructs were used. Data are expressed as percentage of relative MyD88-induced NF-kB activity.

Figures 7A-B show that MyD88 (106-296) selectively inhibits IL-lRs- but not TNFR2-induced NF-kB activity. TRAF6 (298-522) and the related TRAF2 (87-501) were used as controls. 0.5 µg receptors and increasing amounts of putative dominant negative expression constructs were transfected. Data are expressed as percentage of relative IL-lRs or TNFR2-induced NF-kB activity.

Figures 8A-C show that MyD88 dominant negative version, MyD88 (152-296), abrogates IL-lRs-induced but not IRAK-2-induced NF-kB activity.

Conversely IRAK-2 dominant negative versions, IRAK-2 (1-96) and IRAK-2 (97-590), significantly inhibit both IL-lRs and MyD88-induced NF-kB activity. 0.2 μg of inducer and 0.6 μg of dominant negative expression constructs were used in each transfection. Data are expressed as percentage of relative induced NF-kB activity.

Figure 9 is a schematic representation of the molecular order of mediators of the IL-1Rs-induced NF-kB activation.

Figure 10 shows an analysis of the IRAK-2α amino acid sequence. Alpha, beta, turn and coil regions; hydrophilicity and hydrophobicity; amphipathic regions; flexible regions; antigenic index and surface probability are shown. In the "Antigenic Index - Jameson-Wolf" graph, amino acid residues about 96 to about 193, about 207 to about 254, about 293 to about 316, about 416 to about 472, and about 487 to about 541 in Figure 1 (SEQ ID NO:2) correspond to the shown highly antigenic regions of the IRAK-2α protein.

Figure 11 shows an analysis of the IRAK-2 β amino acid sequence. Alpha, beta, turn and coil regions; hydrophilicity and hydrophobicity; amphipathic regions; flexible regions; antigenic index and surface probability are shown. In the "Antigenic Index - Jameson-Wolf" graph, amino acid residues 96 to about 193, about 207 to about 254, about 293 to about 316, about 416 to about 472, about 487 to about 541, and about 559 to about 619 in Figure 2 (SEQ ID NO:4) correspond to the shown highly antigenic regions of the IRAK-2β protein.

Detailed Description

The present inventors have identified a human IRAK-2, IRAK-2α, and a splice variant thereof, IRAK-2β. Thus, the present invention provides isolated nucleic acid molecules comprising a polynucleotide encoding an IRAK-2 polypeptide having the amino acid sequence shown in SEQ ID NO:2. The present invention also provides isolated nucleic acid molecules comprising a polynucleotide encoding an IRAK-2 polypeptide having the amino acid sequence

shown in SEQ ID NO:4, which was determined by sequencing a cloned cDNA. The IRAK-2α and IRAK-2β proteins of the present invention shares sequence homology with IRAK (SEQ ID NO:5) and Pelle (SEQ ID NO:6). The nucleotide sequence shown in SEQ ID NO:3 was obtained by sequencing a cDNA clone, which was deposited on October 7, 1997 at the American Type Culture Collection, 12301 Park Lawn Drive, Rockville, Maryland 20852, and given accession number 209340. The deposited clone is inserted in the pBluescript SK(-) plasmid (Stratagene, LaJolla, CA) using the EcoRI and XhoI restriction endonuclease cleavage sites.

Nucleic Acid Molecules

Unless otherwise indicated, all nucleotide sequences determined by sequencing a DNA molecule herein were determined using an automated DNA sequencer, and all amino acid sequences of polypeptides encoded by DNA molecules determined herein were predicted by translation of a DNA sequence determined as above. Therefore, as is known in the art for any DNA sequence determined by this automated approach, any nucleotide sequence determined herein may contain some errors. Nucleotide sequences determined by automation are typically at least about 90% identical, more typically at least about 95% to at least about 99.9% identical to the actual nucleotide sequence of the sequenced DNA molecule. The actual sequence can be more precisely determined by other approaches including manual DNA sequencing methods well known in the art. As is also known in the art, a single insertion or deletion in a determined nucleotide sequence compared to the actual sequence will cause a frame shift in translation of the nucleotide sequence such that the predicted amino acid sequence encoded by a determined nucleotide sequence will be completely different from the amino acid sequence actually encoded by the sequenced DNA molecule, beginning at the point of such an insertion or deletion.

Using the information provided herein, such as the nucleotide sequence in SEQ ID NO: 1 or SEQ ID NO:3, a nucleic acid molecule of the present invention encoding an IRAK-2 polypeptide may be obtained using standard cloning and screening procedures, such as those for cloning cDNAs using mRNA as starting material. Illustrative of the invention, the nucleic acid molecule described in SEQ ID NO:1 was discovered in a cDNA library derived from HUVEC cells. The determined nucleotide sequence of the IRAK-2 cDNA of SEQ ID NO:1 contains an open reading frame encoding a protein of about 590 amino acid residues and a deduced molecular weight of about 65 kDa. The nucleic acid molecule described in SEQ ID NO:3 was discovered in cDNA libraries derived from HUVEC cells and activated neutrophils. The determined nucleotide sequence of the IRAK-2 cDNA of SEQ ID NO:3 contains an open reading frame encoding a protein of about 625 amino acids. The IRAK-2 proteins shown in SEQ ID NO:2 and SEQ ID NO:4 are about 35-40 % identical and about 50-60 % similar to IRAK (SEQ ID NO:5).

As one of ordinary skill would appreciate, due to the possibilities of sequencing errors, the predicted IRAK-2 polypeptide encoded by the deposited cDNA comprises about 625 amino acids, but may be anywhere in the range of 600-650 amino acids.

As indicated, nucleic acid molecules of the present invention may be in the form of RNA, such as mRNA, or in the form of DNA, including, for instance, cDNA and genomic DNA obtained by cloning or produced synthetically. The DNA may be double-stranded or single-stranded. Single-stranded DNA or RNA may be the coding strand, also known as the sense strand, or it may be the non-coding strand, also referred to as the anti-sense strand.

By "isolated" nucleic acid molecule(s) is intended a nucleic acid molecule, DNA or RNA, which has been removed from its native environment. For example, recombinant DNA molecules contained in a vector are considered isolated for the purposes of the present invention. Further examples of isolated DNA molecules include recombinant DNA molecules maintained in heterologous

host cells or purified (partially or substantially) DNA molecules in solution. Isolated RNA molecules include *in vivo* or *in vitro* RNA transcripts of the DNA molecules of the present invention. Isolated nucleic acid molecules according to the present invention further include such molecules produced synthetically.

Isolated nucleic acid molecules of the present invention include DNA molecules comprising an open reading frame (ORF) shown in SEQ ID NO:1 or SEQ ID NO:3; and DNA molecules which comprise a sequence substantially different from those described above but which, due to the degeneracy of the genetic code, still encode an IRAK-2 protein. Of course, the genetic code is well known in the art. Thus, it would be routine for one skilled in the art to generate such degenerate variants.

In addition, the present inventors have identified the following cDNA clones related to extensive portions of SEQ ID NO:1 and SEQ ID NO:3: HPMCW18R (SEQ ID NO:7), HTADQ88R (SEQ ID NO:8), HNFEL57R (SEQ ID NO:9), HAPCM54R (SEQ ID NO:10), HNFFX36R (SEQ ID NO:11), HNFHL91R (SEQ ID NO:12), and HCE5L53R (SEQ ID NO:13).

The following public EST, which relates to portions of SEQ ID NO:1 and SEQ ID NO:3, has also been identified: Genbank Accession No. N52479, (SEQ ID NO:14).

In another aspect, the invention provides isolated nucleic acid molecules encoding the IRAK-2 polypeptide having an amino acid sequence as encoded by the cDNA clone contained in the plasmid deposited as ATCC Deposit No. 209340 on October 7, 1997. In a further embodiment, nucleic acid molecules are provided encoding the full-length IRAK-2α or IRAK-2β polypeptide lacking the N-terminal methionine. The invention also provides an isolated nucleic acid molecule having the nucleotide sequence shown in SEQ ID NO:1 or SEQ ID NO:3 or the nucleotide sequence of the IRAK-2 cDNA contained in the above-described deposited clone, or a nucleic acid molecule having a sequence complementary to one of the above sequences. Such isolated molecules, particularly DNA molecules, are useful as probes for gene mapping, by *in situ* hybridization with

chromosomes, and for detecting expression of the IRAK-2 gene in human tissue, for instance, by Northern blot analysis.

The present invention is further directed to fragments of the isolated nucleic acid molecules described herein. By a fragment of an isolated nucleic acid molecule having the nucleotide sequence of the deposited cDNA or the nucleotide sequence shown in SEQ ID NO:1 or SEQ ID NO:3 is intended fragments at least about 15 nt, and more preferably at least about 20 nt, still more preferably at least about 30 nt, and even more preferably, at least about 40 nt in length which are useful as diagnostic probes and primers as discussed herein. Of course, larger fragments 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, 1150, 1200, 1250, 1300, 1350, 1400, 1450, 1500, 1550, 1600, 1650, or 1700 nt in length are also useful according to the present invention as are fragments corresponding to most, if not all, of the nucleotide sequence of the deposited cDNA or as shown in SEQ ID NO:1 or SEQ ID NO:3. By a fragment at least 20 nt in length, for example, is intended fragments which include 20 or more contiguous bases from the nucleotide sequence of the deposited cDNA or the nucleotide sequence as shown in SEQ ID NO:1 or SEQ ID NO:3.

Preferred nucleic acid fragments of the present invention include nucleic acid molecules encoding epitope-bearing portions of the IRAK-2 protein. In particular, such nucleic acid fragments of the present invention include nucleic acid molecules encoding: a polypeptide comprising amino acid residues from about 96 to about 193 in SEQ ID NO:2 or SEQ ID NO:4; a polypeptide comprising amino acid residues from about 207 to about 254 in SEQ ID NO:2 or SEQ ID NO:4; a polypeptide comprising amino acid residues from about 293 to about 316 in SEQ ID NO:2 or SEQ ID NO: 4; a polypeptide comprising amino acid residues from about 416 to about 472 in SEQ ID NO:2 or SEQ ID NO:4; a polypeptide comprising amino acid residues from about 487 to about 541 in SEQ ID NO:2 or SEQ ID NO:4; and a polypeptide comprising amino acid residues from about 559 to about 619 in SEQ ID NO:4. The inventors have determined that the above

polypeptide fragments are antigenic regions of the IRAK-2 polypeptides. Methods for determining other such epitope-bearing portions of the IRAK-2 protein are described in detail below.

In another aspect, the invention provides an isolated nucleic acid molecule comprising a polynucleotide which hybridizes under stringent hybridization conditions to a portion of the polynucleotide in a nucleic acid molecule of the invention described above, for instance, the cDNA clone contained in ATCC Deposit 209340. By "stringent hybridization conditions" is intended overnight incubation at 42°C in a solution comprising: 50% formamide, 5x SSC (150 mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 g/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1x SSC at about 65°C.

By a polynucleotide which hybridizes to a "portion" of a polynucleotide is intended a polynucleotide (either DNA or RNA) hybridizing to at least about 15 nucleotides (nt), and more preferably at least about 20 nt, still more preferably at least about 30 nt, and even more preferably about 30-70 nt of the reference polynucleotide. These are useful as diagnostic probes and primers as discussed above and in more detail below.

By a portion of a polynucleotide of "at least 20 nt in length," for example, is intended 20 or more contiguous nucleotides from the nucleotide sequence of the reference polynucleotide (e.g., the deposited cDNA or the nucleotide sequence as shown in SEQ ID NO:1 or SEQ ID NO:3). Of course, a polynucleotide which hybridizes only to a poly A sequence (such as the 3′ terminal poly(A) tract of the IRAK-2 cDNA shown in SEQ ID NO:1 or SEQ ID NO:3), or to a complementary stretch of T (or U) resides, would not be included in a polynucleotide of the invention used to hybridize to a portion of a nucleic acid of the invention, since such a polynucleotide would hybridize to any nucleic acid molecule containing a poly (A) stretch or the complement thereof (e.g., practically any double-stranded cDNA clone).

As indicated, nucleic acid molecules of the present invention which encode an IRAK-2 polypeptide may include, but are not limited to those encoding the amino acid sequence of the full-length polypeptide, by itself; the coding sequence for the full-length polypeptide and additional sequences, such as those encoding a leader or secretory sequence, such as a pre-, or pro- or prepro- protein sequence; the coding sequence of the full-length polypeptide, with or without the aforementioned additional coding sequences, together with additional, non-coding sequences, including for example, but not limited to introns and non-coding 5′ and 3′ sequences, such as the transcribed, non-translated sequences that play a role in transcription, mRNA processing, including splicing and polyadenylation signals, for example - ribosome binding and stability of mRNA; an additional coding sequence which codes for additional amino acids, such as those which provide additional functionalities. Thus, the sequence encoding the polypeptide may be fused to a marker sequence, such as a sequence encoding a peptide which facilitates purification of the fused polypeptide. In certain preferred embodiments of this aspect of the invention, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (Qiagen, Inc.), among others, many of which are commercially available. As described in Gentz *et al.*, *Proc. Natl. Acad. Sci. USA 86*:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. The "HA" tag is another peptide useful for purification which corresponds to an epitope derived from the influenza hemagglutinin protein, which has been described by Wilson *et al.*, *Cell 37*:767-778 (1984). As discussed below, other such fusion proteins include the IRAK-2 fused to Fc at the N- or C-terminus.

The present invention further relates to variants of the nucleic acid molecules of the present invention, which encode portions, analogs or derivatives of the IRAK-2 protein. Variants may occur naturally, such as a natural allelic variant. By an "allelic variant" is intended one of several alternate forms of a gene occupying a given locus on a chromosome of an organism. *Genes II*, Lewin, B.,

ed., John Wiley & Sons, New York (1985). Non-naturally occurring variants may be produced using art-known mutagenesis techniques.

Such variants include those produced by nucleotide substitutions, deletions or additions, which may involve one or more nucleotides. The variants may be altered in coding regions, non-coding regions, or both. Alterations in the coding regions may produce conservative or non-conservative amino acid substitutions, deletions or additions. Especially preferred among these are silent substitutions, additions and deletions, which do not alter the properties and activities of the IRAK-2 protein or portions thereof. Also especially preferred in this regard are conservative substitutions.

Further embodiments of the invention include isolated nucleic acid molecules comprising a polynucleotide having a nucleotide sequence at least 95% identical, and more preferably at least 96%, 97%, 98% or 99% identical to (a) a nucleotide sequence encoding the polypeptide having the amino acid sequence in SEQ ID NO:2; (b) a nucleotide sequence encoding the polypeptide having the amino acid sequence in SEQ ID NO:2, but lacking the N-terminal methionine; (c) a nucleotide sequence encoding the polypeptide having the amino acid sequence in SEQ ID NO:4; (d) a nucleotide sequence encoding the polypeptide having the amino acid sequence in SEQ ID NO:4, but lacking the N-terminal methionine; (e) a nucleotide sequence encoding the polypeptide having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 209340; (f) a nucleotide sequence encoding the IRAK-2 polypeptide having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 209340, but lacking the N-terminal methionine; or (g) a nucleotide sequence complementary to any of the nucleotide sequences in (a), (b), (c), (d), (e), or (f).

By a polynucleotide having a nucleotide sequence at least, for example, 95% "identical" to a reference nucleotide sequence encoding a IRAK-2 polypeptide is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference

nucleotide sequence encoding the IRAK-2 polypeptide. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. These mutations of the reference sequence may occur at the 5′ or 3′ terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence.

As a practical matter, whether any particular nucleic acid molecule is at least 95%, 96%, 97%, 98% or 99% identical to, for instance, the nucleotide sequence shown in SEQ ID NO:1 or SEQ ID NO:3 or to the nucleotides sequence of the deposited cDNA clone can be determined conventionally using known computer programs such as the Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711). Bestfit uses the local homology algorithm of Smith and Waterman, *Advances in Applied Mathematics 2*: 482-489 (1981), to find the best segment of homology between two sequences. When using Bestfit or any other sequence alignment program to determine whether a particular sequence is, for instance, 95% identical to a reference sequence according to the present invention, the parameters are set, of course, such that the percentage of identity is calculated over the full length of the reference nucleotide sequence and that gaps in homology of up to 5% of the total number of nucleotides in the reference sequence are allowed.

The present application is directed to nucleic acid molecules at least 95%, 96%, 97%, 98% or 99% identical to the nucleic acid sequence shown in SEQ ID NO:1 or SEQ ID NO:3 or to the nucleic acid sequence of the deposited cDNA, irrespective of whether they encode a polypeptide having IRAK-2 activity. This is because even where a particular nucleic acid molecule does not encode a

polypeptide having IRAK-2 activity, one of skill in the art would still know how to use the nucleic acid molecule, for instance, as a hybridization probe or a polymerase chain reaction (PCR) primer. Uses of the nucleic acid molecules of the present invention that do not encode a polypeptide having IRAK-2 activity include, *inter alia*, (1) isolating the IRAK-2 gene or allelic variants thereof in a cDNA library; (2) *in situ* hybridization (e.g., "FISH") to metaphase chromosomal spreads to provide precise chromosomal location of the IRAK-2 gene, as described in Verma *et al.*, *Human Chromosomes: A Manual of Basic Techniques*, Pergamon Press, New York (1988); and (3) Northern Blot analysis for detecting IRAK-2 mRNA expression in specific tissues.

Preferred, however, are nucleic acid molecules having sequences at least 95%, 96%, 97%, 98% or 99% identical to a nucleic acid sequence shown in SEQ ID NO:1 or SEQ ID NO:3 or to a nucleic acid sequence of the deposited cDNA which do, in fact, encode a polypeptide having IRAK-2 protein activity. By "a polypeptide having IRAK-2 activity" is intended polypeptides exhibiting IRAK-2 activity in a particular biological assay. For example, IRAK-2 protein activity can be measured using the luciferase assay described in Cao *et al.*, *Nature 383*: 443-446 (1996) and below in Example 1.

Briefly, cells which have been transfected with a nucleic acid encoding for a candidate polypeptide, such as human 293 cells, are transfected with an ELAM-1-luciferase reporter plasmid. Luciferase activity is measured in these cells and compared to cells which have been transfected with the luciferase construct, but not with the candidate polypeptide. A higher level of luciferase activity in cells with the candidate polypeptide is indicative of IRAK-2 activity.

Of course, due to the degeneracy of the genetic code, one of ordinary skill in the art will immediately recognize that a large number of the nucleic acid molecules having a sequence at least 95%, 96%, 97%, 98%, or 99% identical to a nucleic acid sequence of the deposited cDNA or a nucleic acid sequence shown in SEQ ID NO:1 or SEQ ID NO:3 will encode a polypeptide "having IRAK-2 protein activity." In fact, since degenerate variants of these nucleotide sequences

all encode the same polypeptide, this will be clear to the skilled artisan even without performing the above described comparison assay. It will be further recognized in the art that, for such nucleic acid molecules that are not degenerate variants, a reasonable number will also encode a polypeptide having IRAK-2 protein activity. This is because the skilled artisan is fully aware of amino acid substitutions that are either less likely or not likely to significantly effect protein function (e.g., replacing one aliphatic amino acid with a second aliphatic amino acid).

For example, guidance concerning how to make phenotypically silent amino acid substitutions is provided in Bowie, J. U. *et al.*, "Deciphering the Message in Protein Sequences: Tolerance to Amino Acid Substitutions," *Science 247:*1306-1310 (1990), wherein the authors indicate that proteins are surprisingly tolerant of amino acid substitutions.

Vectors and Host Cells

The present invention also relates to vectors which include the isolated DNA molecules of the present invention, host cells which are genetically engineered with the recombinant vectors, and the production of IRAK-2 polypeptides or fragments thereof by recombinant techniques.

The polynucleotides may be joined to a vector containing a selectable marker for propagation in a host. Generally, a plasmid vector is introduced in a precipitate, such as a calcium phosphate precipitate, or in a complex with a charged lipid. If the vector is a virus, it may be packaged *in vitro* using an appropriate packaging cell line and then transduced into host cells.

The DNA insert should be operatively linked to an appropriate promoter, such as the phage lambda PL promoter, the *E. coli lac, trp* and *tac* promoters, the SV40 early and late promoters and promoters of retroviral LTRs, to name a few. Other suitable promoters will be known to the skilled artisan. The expression constructs will further contain sites for transcription initiation, termination and, in

the transcribed region, a ribosome binding site for translation. The coding portion of the mature transcripts expressed by the constructs will preferably include a translation initiating at the beginning and a termination codon (UAA, UGA or UAG) appropriately positioned at the end of the polypeptide to be translated.

As indicated, the expression vectors will preferably include at least one selectable marker. Such markers include dihydrofolate reductase or neomycin resistance for eukaryotic cell culture and tetracycline or ampicillin resistance genes for culturing in *E. coli* and other bacteria. Representative examples of appropriate hosts include, but are not limited to, bacterial cells, such as *E. coli*, *Streptomyces* and *Salmonella typhimurium* cells; fungal cells, such as yeast cells; insect cells such as *Drosophila* S2 and *Spodoptera* Sf9 cells; animal cells such as CHO, COS and Bowes melanoma cells; and plant cells. Appropriate culture mediums and conditions for the above-described host cells are known in the art.

Among vectors preferred for use in bacteria include pQE70, pQE60 and pQE-9, available from Qiagen; pBS vectors, Phagescript vectors, Bluescript vectors, pNH8A, pNH16a, pNH18A, pNH46A, available from Stratagene; and ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 available from Pharmacia. Among preferred eukaryotic vectors are pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; and pSVK3, pBPV, pMSG and pSVL available from Pharmacia. Other suitable vectors will be readily apparent to the skilled artisan.

Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection or other methods. Such methods are described in many standard laboratory manuals, such as Davis *et al.*, *Basic Methods In Molecular Biology* (1986).

The polypeptide may be expressed in a modified form, such as a fusion protein, and may include not only secretion signals, but also additional heterologous functional regions. For instance, a region of additional amino acids, particularly charged amino acids, may be added to the N-terminus of the

polypeptide to improve stability and persistence in the host cell, during purification, or during subsequent handling and storage. Also, peptide moieties may be added to the polypeptide to facilitate purification. Such regions may be removed prior to final preparation of the polypeptide. The addition of peptide moieties to polypeptides to engender secretion or excretion, to improve stability and to facilitate purification, among others, are familiar and routine techniques in the art. A preferred fusion protein comprises a heterologous region from immunoglobulin that is useful to solubilize proteins. For example, EP-A-O 464 533 (Canadian counterpart 2045869) discloses fusion proteins comprising various portions of constant region of immunoglobin molecules together with another human protein or part thereof. In many cases, the Fc part in a fusion protein is thoroughly advantageous for use in therapy and diagnosis and thus results, for example, in improved pharmacokinetic properties (EP-A 0232 262). On the other hand, for some uses it would be desirable to be able to delete the Fc part after the fusion protein has been expressed, detected and purified in the advantageous manner described. This is the case when Fc portion proves to be a hindrance to use in therapy and diagnosis, for example when the fusion protein is to be used as antigen for immunizations. In drug discovery, for example, human proteins, such as, hIL5-receptor has been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. See, D. Bennett *et al.*, *Journal of Molecular Recognition*, Vol. 8:52-58 (1995) and K. Johanson *et al.*, *The Journal of Biological Chemistry*, Vol. 270, No. 16:9459-9471 (1995).

The IRAK-2 protein can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography ("HPLC") is employed for purification. Polypeptides of the present invention

include naturally purified products, products of chemical synthetic procedures, and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant, insect and mammalian cells. Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. In addition, polypeptides of the invention may also include an initial modified methionine residue, in some cases as a result of host-mediated processes.

IRAK-2 Polypeptides and Fragments

The invention further provides an isolated IRAK-2 polypeptide having the amino acid sequence encoded by the deposited cDNA, or the amino acid sequence in SEQ ID NO:2, or the amino acid sequence in SEQ ID NO:4, or a peptide or polypeptide comprising a portion of the above polypeptides.

It will be recognized in the art that some amino acid sequences of the IRAK-2α or IRAK-2β polypeptides can be varied without significant effect of the structure or function of the protein. If such differences in sequence are contemplated, it should be remembered that there will be critical areas on the protein which determine activity.

Thus, the invention further includes variations of the IRAK-2α or IRAK-2β polypeptide which show substantial IRAK-2 polypeptide activity or which include regions of IRAK-2 protein such as the protein portions discussed below. Such mutants include deletions, insertions, inversions, repeats, and type substitutions. As indicated above, guidance concerning which amino acid changes are likely to be phenotypically silent can be found in Bowie, J.U., *et al.*, "Deciphering the Message in Protein Sequences: Tolerance to Amino Acid Substitutions," *Science 247:*1306-1310 (1990).

Thus, the fragment, derivative or analog of the polypeptide of SEQ ID NO:2 or SEQ ID NO:4, or that encoded by the deposited cDNA, may be (i) one

in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a substituent group, or (iii) one in which the mature polypeptide is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol), or (iv) one in which the additional amino acids are fused to the mature polypeptide, such as an IgG Fc fusion region peptide or leader or secretory sequence or a sequence which is employed for purification of the mature polypeptide or a proprotein sequence. Such fragments, derivatives and analogs are deemed to be within the scope of those skilled in the art from the teachings herein.

Of particular interest are substitutions of charged amino acids with another charged amino acid and with neutral or negatively charged amino acids. The latter results in proteins with reduced positive charge to improve the characteristics of the IRAK-2 protein. The prevention of aggregation is highly desirable. Aggregation of proteins not only results in a loss of activity but can also be problematic when preparing pharmaceutical formulations, because they can be immunogenic. (Pinckard *et al.*, *Clin. Exp. Immunol.* 2:331-340 (1967); Robbins *et al.*, *Diabetes* 36:838-845 (1987); Cleland *et al. Crit. Rev. Therapeutic Drug Carrier Systems* 10:307-377 (1993)).

As indicated, changes are preferably of a minor nature, such as conservative amino acid substitutions that do not significantly affect the folding or activity of the protein (see Table 1).

TABLE 1. Conservative Amino Acid Substitutions.

Aromatic	Phenylalanine Tryptophan Tyrosine
Hydrophobic	Leucine Isoleucine Valine
Polar	Glutamine Asparagine
Basic	Arginine Lysine Histidine
Acidic	Aspartic Acid Glutamic Acid
Small	Alanine Serine Threonine Methionine Glycine

Of course, the number of amino acid substitutions a skilled artisan would make depends on many factors, including those described above. Generally speaking, the number of amino acid substitutions for any given IRAK-2 polypeptide will not be more than 50, 40, 30, 20, 10, 5 or 3.

Amino acids in the IRAK-2 proteins of the present invention that are essential for function can be identified by methods known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (Cunningham and Wells, *Science 244*:1081-1085 (1989)). The latter procedure introduces single alanine mutations at every residue in the molecule. The resulting mutant molecules are then tested for biological activity such as *in vitro* proliferative activity.

The polypeptides of the present invention are preferably provided in an isolated form. By "isolated polypeptide" is intended a polypeptide removed from its native environment. Thus, a polypeptide produced and/or contained within a recombinant host cell is considered isolated for purposes of the present invention.

Also intended as an "isolated polypeptide" are polypeptides that have been purified, partially or substantially, from a recombinant host cell or a native source. For example, a recombinantly produced version of the IRAK-2 polypeptide can be substantially purified by the one-step method described in Smith and Johnson, *Gene 67*:31-40 (1988).

The polypeptides of the present invention include the a polypeptide comprising the polypeptide encoded by the deposited cDNA; a polypeptide comprising the polypeptide encoded by the deposited cDNA, but minus the N-terminal methionine; a polypeptide comprising amino acids about 1 to about 590 in SEQ ID NO:2; a polypeptide comprising amino acids about 2 to about 590 in SEQ ID NO:2; a polypeptide comprising amino acids about 1 to about 625 in SEQ ID NO:4; a polypeptide comprising amino acids about 2 to about 625 in SEQ ID NO:4; as well as polypeptides which are at least 95% identical, and more preferably at least 96%, 97%, 98% or 99% identical to those described above and also include portions of such polypeptides with at least 30 amino acids and more preferably at least 50 amino acids.

By a polypeptide having an amino acid sequence at least, for example, 95% "identical" to a reference amino acid sequence of a IRAK-2 polypeptide is intended that the amino acid sequence of the polypeptide is identical to the reference sequence except that the polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the reference amino acid of the IRAK-2 polypeptide. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a reference amino acid sequence, up to 5% of the amino acid residues in the reference sequence may be deleted or substituted with another amino acid, or a number of amino acids up to 5% of the total amino acid residues in the reference sequence may be inserted into the reference sequence. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues

in the reference sequence or in one or more contiguous groups within the reference sequence.

As a practical matter, whether any particular polypeptide is at least 95%, 96%, 97%, 98% or 99% identical to, for instance, the amino acid sequence shown in SEQ ID NO:2 or SEQ ID NO:4 or to the amino acid sequence encoded by deposited cDNA clone can be determined conventionally using known computer programs such the Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711). When using Bestfit or any other sequence alignment program to determine whether a particular sequence is, for instance, 95% identical to a reference sequence according to the present invention, the parameters are set, of course, such that the percentage of identity is calculated over the full length of the reference amino acid sequence and that gaps in homology of up to 5% of the total number of amino acid residues in the reference sequence are allowed.

The polypeptide of the present invention are useful as a molecular weight marker on SDS-PAGE gels or on molecular sieve gel filtration columns using methods well known to those of skill in the art.

In another aspect, the invention provides a peptide or polypeptide comprising an epitope-bearing portion of a polypeptide of the invention. The epitope of this polypeptide portion is an immunogenic or antigenic epitope of a polypeptide described herein. An "immunogenic epitope" is defined as a part of a protein that elicits an antibody response when the whole protein is the immunogen. On the other hand, a region of a protein molecule to which an antibody can bind is defined as an "antigenic epitope." The number of immunogenic epitopes of a protein generally is less than the number of antigenic epitopes. See, for instance, Geysen *et al.*, *Proc. Natl. Acad. Sci. USA 81*:3998-4002 (1983).

As to the selection of peptides or polypeptides bearing an antigenic epitope (i.e., that contain a region of a protein molecule to which an antibody can bind),

it is well known in that art that relatively short synthetic peptides that mimic part of a protein sequence are routinely capable of eliciting an antiserum that reacts with the partially mimicked protein. See, for instance, Sutcliffe, J. G., Shinnick, T. M., Green, N. and Learner, R.A. (1983) Antibodies that react with predetermined sites on proteins. *Science 219:*660-666. Peptides capable of eliciting protein-reactive sera are frequently represented in the primary sequence of a protein, can be characterized by a set of simple chemical rules, and are confined neither to immunodominant regions of intact proteins (i.e., immunogenic epitopes) nor to the amino or carboxyl terminals.

Antigenic epitope-bearing peptides and polypeptides of the invention are therefore useful to raise antibodies, including monoclonal antibodies, that bind specifically to a polypeptide of the invention. See, for instance, Wilson *et al., Cell 37*:767-778 (1984) at 777.

Antigenic epitope-bearing peptides and polypeptides of the invention preferably contain a sequence of at least seven, more preferably at least nine and most preferably between about at least about 15 to about 30 amino acids contained within the amino acid sequence of a polypeptide of the invention.

Non-limiting examples of antigenic polypeptides or peptides that can be used to generate IRAK-2-specific antibodies include: a polypeptide comprising amino acid residues from about 96 to about 193 in SEQ ID NO:2 or SEQ ID NO:4; a polypeptide comprising amino acid residues from about 207 to about 254 in SEQ ID NO:2 OR SEQ ID NO:4; a polypeptide comprising amino acid residues from about 293 to about 316 in SEQ ID NO:2 or SEQ ID NO: 4; a polypeptide comprising amino acid residues from about 416 to about 472 in SEQ ID NO:2 or SEQ ID NO:4; a polypeptide comprising amino acid residues from about 487 to about 541 in SEQ ID NO:2 or SEQ ID NO:4; and a polypeptide comprising amino acid residues from about 559 to about 619 in SEQ ID NO:4. As indicated above, the inventors have determined that the above polypeptide fragments are antigenic regions of the IRAK-2 protein.

The epitope-bearing peptides and polypeptides of the invention may be produced by any conventional means. Houghten, R. A. (1985) General method for the rapid solid-phase synthesis of large numbers of peptides: specificity of antigen-antibody interaction at the level of individual amino acids. *Proc. Natl. Acad. Sci. USA 82*:5131-5135. This "Simultaneous Multiple Peptide Synthesis (SMPS)" process is further described in U.S. Patent No. 4,631,211 to Houghten *et al.* (1986).

As one of skill in the art will appreciate, IRAK-2 polypeptides of the present invention and the epitope-bearing fragments thereof described above can be combined with parts of the constant domain of immunoglobulins (IgG), resulting in chimeric polypeptides. These fusion proteins facilitate purification and show an increased half-life *in vivo*. This has been shown, e.g., for chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins (EPA 394,827; Traunecker *et al.*, *Nature 331*:84-86 (1988)). Fusion proteins that have a disulfide-linked dimeric structure due to the IgG part can also be more efficient in binding and neutralizing other molecules than the monomeric IRAK-2 protein or protein fragment alone (Fountoulakis *et al.*, *J. Biochem 270*:3958-3964 (1995)).

Screening Assays

The present inventors have shown that IRAK-2 mediates NF-kB activation induced by IL-1R stimulation. NF-kB is an ubiquitous transcription factor which has been shown to activate transcription of enzymes, such as cyclooxygenase-2 (Newton *et al.*, *Biochem. Biophys. Res. Commun. 237(1)*:28-32 (1997)); cytokines, such as RANTES (Moriuchi *et al.*, *J. Immunol. 158(7)*:3483-3491 (1997)); adhesion molecules, such as E-selectin (ELAM-1) (Read *et al.*, *J. Biol. Chem. 272(5)*:2753-2761 (1997)); and other molecules. The normal functions of NF-kB include communication between cells, embryonal development, the

response to stress, inflammation and viral infection, and the maintenance of cell type specific expression of genes (for review, see Wulczyn *et al.*, *J. Mol. Med. 74(12)*:749-769 (1996)). Upregulation of NF-kB could be used to treat viral infections, such as HIV ((Moriuchi *et al.*, *J. Immunol. 158(7)*:3483-3491 (1997)), and damage caused by oxidative stress (Renard *et al.*, *Biochem. Pharmacol. 53*:149-160 (1997)). Disregulation of NF-kB activation has been linked to adult respiratory distress syndrome, sepsis syndrome, asthma, rheumatoid arthritis, inflammatory bowel disease, malignant transformation and hyperplasia (Blackwell *et al.*, *Am. J. Respir. Cell. Mol. Biol. 17(1)*:3-9 (1997); Barnes, *Int. J. Biochem. Cell. Biol. 29(6)*:867-870 (1997); and Gilmore *et al.*, *Oncogene 9*:2391-2398 (1996)). Accordingly, inhibitors of NF-kB could be used to treat these disorders. Several inhibitors of NF-kB have been identified, including antioxidants such as alpha-tocopherol (Erl *et al.*, *Am. J. Physiol. 273*:H634-H640 (1997)), and glucocorticoids, such as dexamethasone (Wang *et al.*, *J. Immunol. 159*:534-537 (1997))).

Thus, the present invention also provides a screening method for determining whether a compound of interest is an agonist or antagonist of the IRAK-2 pathway. This method involves contacting cells which express IRAK-2, either exogenously or endogenously, with a compound of interest, assaying NF-kB mediated transcription, and comparing the NF-kB mediated transcription to a standard response. The standard response is the level of NF-kB mediated transcription in cells expressing IRAK-2 that have not been contacted with the compound of interest, whereby an increase in NF-kB mediated transcription over the standard indicates that the compound of interest is an agonist of the IRAK-2 pathway and a decrease in NF-kB mediated transcription under the standard indicates that the compound of interest is an antagonist of the IRAK-2 pathway.

By "assaying NF-kB mediated transcription" is intended qualitatively or quantitatively measuring NF-kB mediated transcription. By the invention, the compound of interest is an agonist of the IRAK-2 pathway if NF-kB mediated transcription is enhanced over that observed due to IRAK-2 in the absence of the

compound of interest and the compound of interest is an antagonist of the IRAK-2 pathway if NF-kB mediated transcription is diminished compared to that observed due to IRAK-2 in the absence of the compound of interest. Since IRAK-2 activates NF-kB transcription, any *in vitro* or *in vivo* assay which measures NF-kB activity can be used in this method.

For example, a construct encoding for IRAK-2 is transfected into a cell, along with a construct containing a reporter gene which is under the control of a promoter which is activated in the presence of NF-kB. Any reporter gene which is known in the art can be used in this assay. Examples of reporter genes useful in this assay include, but are not limited to, luciferase, β-galactosidase, and chloramphenicol acetyltransferase. NF-kB-responsive promoters can include one or more binding sites for NF-kB. Examples of promoters which are sensitive to NF-kB include, but are not limited to, the promoter for ELAM-1 and the promoter for RANTES. After transfection of the constructs, the cell is contacted with a compound of interest, and the reporter gene expression is measured and compared to the reporter gene expression seen in cells which have not been contacted with the compound of interest. An increase in reporter gene expression in cells which have been contacted with the compound of interest indicates that the compound is an agonist of the IRAK-2 pathway. A decrease in reporter gene expression in cells which have been contacted with the compound of interest indicates that the compound is an antagonist of the IRAK-2 pathway.

IRAK-2 Related Disorder Diagnosis

For IRAK-2 related disorders, it is believed that substantially altered (increased or decreased) levels of IRAK-2 gene expression can be detected in tissues taken from a mammal having such a disorder, relative to a "standard" mammal, i.e., a mammal of the same species not having the disorder. Thus, the invention provides a diagnostic method useful during diagnosis of an IRAK-2 related disorder, which involves assaying the expression level of the gene encoding

the IRAK-2 protein in mammalian cells or body fluid and comparing the gene expression level with a standard IRAK-2 gene expression level, whereby an increase in the gene expression level over the standard is indicative of certain disorders.

IRAK-2 related disorders are believe to include, but are not limited to, leukemia, lymphoma, rheumatoid arthritis, sarcoidosis, tuberculosis, onchocerciasis, allergies, various bacterial infections, arteriosclerosis, autoimmune diseases, and inflammatory diseases.

Where a diagnosis has already been made according to conventional methods, the present invention is useful as a prognostic indicator, whereby patients exhibiting enhanced IRAK-2 gene expression will experience a worse clinical outcome relative to patients expressing the gene at a lower level.

By "assaying the expression level of the gene encoding the IRAK-2 protein" is intended qualitatively or quantitatively measuring or estimating the level of the IRAK-2 protein or the level of the mRNA encoding the IRAK-2 protein in a first biological sample either directly (e.g., by determining or estimating absolute protein level or mRNA level) or relatively (e.g., by comparing to the IRAK-2 protein level or mRNA level in a second biological sample).

Preferably, the IRAK-2 protein level or mRNA level in the first biological sample is measured or estimated and compared to a standard IRAK-2 protein level or mRNA level, the standard being taken from a second biological sample obtained from an individual not having the disorder. As will be appreciated in the art, once a standard IRAK-2 protein level or mRNA level is known, it can be used repeatedly as a standard for comparison.

By "biological sample" is intended any biological sample obtained from an individual, cell line, tissue culture, or other source which contains IRAK-2 protein or mRNA. Biological samples include mammalian body fluids (such as sera, plasma, urine, synovial fluid and spinal fluid) which contain IRAK-2 protein, and ovarian, prostate, heart, placenta, pancreas liver, spleen, lung, breast and umbilical tissue.

Preferred mammals include monkeys, apes, cats, dogs, cows, pigs, horses, rabbits and humans. Particularly preferred are humans.

Total cellular RNA can be isolated from a biological sample using the single-step guanidinium-thiocyanate-phenol-chloroform method described in Chomczynski and Sacchi, *Anal. Biochem. 162:*156-159 (1987). Levels of mRNA encoding the IRAK-2 protein are then assayed using any appropriate method. These include Northern blot analysis (Harada *et al., Cell 63:*303-312 (1990)), S1 nuclease mapping (Fujita *et al., Cell 49:*357- 367 (1987)), the polymerase chain reaction (PCR), reverse transcription in combination with the polymerase chain reaction (RT-PCR) (Makino *et al., Technique 2:*295-301 (1990)), and reverse transcription in combination with the ligase chain reaction (RT-LCR).

Assaying IRAK-2 protein levels in a biological sample can occur using antibody-based techniques. For example, IRAK-2 protein expression in tissues can be studied with classical immunohistological methods (Jalkanen, M., *et al., J. Cell. Biol. 101:*976-985 (1985); Jalkanen, M., *et al., J. Cell . Biol. 105:*3087-3096 (1987)).

Other antibody-based methods useful for detecting IRAK-2 protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA).

Suitable labels are known in the art and include enzyme labels, such as, Glucose oxidase, and radioisotopes, such as iodine (^{125}I, ^{121}I), carbon (^{14}C), sulfur (^{35}S), tritium (^{3}H), indium (^{112}In), and technetium (^{99m}Tc), and fluorescent labels, such as fluorescein and rhodamine, and biotin.

Modes of administration

It will be appreciated that conditions caused by a decrease in the standard or normal level of IRAK-2 activity in an individual can be treated by administration of IRAK-2 protein. Thus, the invention further provides a method

of treating an individual in need of an increased level of IRAK-2 activity comprising administering to such an individual a pharmaceutical composition comprising an effective amount of an isolated IRAK-2 polypeptide of the invention effective to increase the IRAK-2 activity level in such an individual.

As a general proposition, the total pharmaceutically effective amount of IRAK-2 polypeptide administered parenterally per dose will be in the range of about 1 µg/kg/day to 10 mg/kg/day of patient body weight, although, as noted above, this will be subject to therapeutic discretion. More preferably, this dose is at least 0.01 mg/kg/day, and most preferably for humans between about 0.01 and 1 mg/kg/day for the hormone. If given continuously, the IRAK-2 polypeptide is typically administered at a dose rate of about 1 µg/kg/hour to about 50 µg/kg/hour, either by 1-4 injections per day or by continuous subcutaneous infusions, for example, using a mini-pump. An intravenous bag solution may also be employed.

Pharmaceutical compositions containing the IRAK-2 of the invention may be administered orally, rectally, parenterally, intracistemally, intravaginally, intraperitoneally, topically (as by powders, ointments, drops or transdermal patch), bucally, or as an oral or nasal spray. By "pharmaceutically acceptable carrier" is meant a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

Chromosome Assays

The nucleic acid molecules of the present invention are also valuable for chromosome identification. The sequence is specifically targeted to and can hybridize with a particular location on an individual human chromosome. The mapping of DNAs to chromosomes according to the present invention is an

important first step in correlating those sequences with genes associated with disease.

In certain preferred embodiments in this regard, the cDNA herein disclosed is used to clone genomic DNA of a IRAK-2 protein gene. This can be accomplished using a variety of well known techniques and libraries, which generally are available commercially. The genomic DNA then is used for *in situ* chromosome mapping using well known techniques for this purpose.

In addition, in some cases, sequences can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp) from the cDNA. Computer analysis of the 3′ untranslated region of the gene is used to rapidly select primers that do not span more than one exon in the genomic DNA, thus complicating the amplification process. These primers are then used for PCR screening of somatic cell hybrids containing individual human chromosomes.

Fluorescence *in situ* hybridization ("FISH") of a cDNA clone to a metaphase chromosomal spread can be used to provide a precise chromosomal location in one step. This technique can be used with probes from the cDNA as short as 50 or 60 bp. For a review of this technique, see Verma *et al.*, *Human Chromosomes: A Manual Of Basic Techniques*, Pergamon Press, New York (1988).

Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found, for example, in V. McKusick, *Mendelian Inheritance In Man*, available on-line through Johns Hopkins University, Welch Medical Library. The relationship between genes and diseases that have been mapped to the same chromosomal region are then identified through linkage analysis (coinheritance of physically adjacent genes).

Next, it is necessary to determine the differences in the cDNA or genomic sequence between affected and unaffected individuals. If a mutation is observed in some or all of the affected individuals but not in any normal individuals, then the mutation is likely to be the causative agent of the disease.

Having generally described the invention, the same will be more readily understood by reference to the following examples, which are provided by way of illustration and are not intended as limiting.

Examples

Example 1: Characterization of IRAK-2α

A novel partial human cDNA was identified that showed significant homology to both IRAK and Pelle. Screening of a human HUVEC cDNA library resulted in the isolation of a full length cDNA clone; analysis of the nucleotide sequence revealed an open reading frame encoding a 590 amino acids (aa) protein with a calculated MW of 65 kDa (Figure 1). Clustall alignment analysis showed significant homology to both IRAK and Pelle (Figure 3). Given its sequence and functional similarity to IRAK the molecule was designated IRAK-2. Northern blot analysis revealed a single IRAK-2 transcript expressed in a variety of tissues whose size (about 4Kbp) was consistent with that of the cDNA.

Ectopic expression of IRAK-2 in human 293 cells induced NF-kB activation as determined by relative luciferase activity of a NF-kB responsive construct. Truncated versions of IRAK-2 encoding amino acid residues 1 to 96 of SEQ ID NO:2 [IRAK-2 (1-96)] or amino acid residues 97 to 590 of SEQ ID NO:2 [IRAK-2 (97-590)] failed to induce any luciferase activity suggesting that integrity of the molecule was essential for its function (Figure 4A). Deletional analysis has previously shown that a mutant version of Pelle analogous to IRAK-2 (97-590) is also inactive leading to the suggestion that Pelle's recruitment to the plasma membrane through its N-terminal domain is necessary for its subsequent function (Galindo, R.L., *et al.*, *Development 121*:2209-2218 (1995)). Given this, it was tested whether IRAK-2 (1-96) or IRAK-2 (97-590) could act as dominant negative inhibitors of IL-1R-induced NF-kB activity. Coexpression of IL-1RI and IL-1RAcP (IL-1Rs for clarity) strongly induced NF-kB activity. Surprisingly, both IRAK-2 (1-96) and IRAK-2 (97-590) inhibited IL-1Rs-induced NF-kB

activity. A dominant negative mutant version of the downstream kinase NIK that is implicated in IL-1R-induced NF-kB activation was used as a positive control; the unrelated adapter molecule TRAF2 (298-522) was used as a negative control (Figure 4B).

Given the sequence similarity shared by IRAK and IRAK-2, and the functional involvement of IRAK-2 in IL-1Rs-induced NF-kB activity, it was analyzed whether IRAK-2 was recruited to the IL-1R signaling complex. Interestingly, while IRAK preferentially coprecipitated with IL-1RAcP, IRAK-2 preferentially bound to the IL-1RI. In contrast, a mutant version of IRAK-2 lacking the first 96 amino acid residues [IRAK-2 (97-590)] failed to associate with IL-1RI suggesting that its N-terminal domain docks with the cytoplasmic domain of IL-1RI. Confirming this was the finding that a truncated form of IRAK-2 coding for the first 96 amino acid residues [IRAK-2 (1-96)] specifically coprecipitated with IL-1RI.

Certain members of the TRAF adapter family mediate NF-kB activation induced by a number of cytokine receptors. TRAF2, for example plays a critical role in TNFR1 and -2 mediated NF-kB activation. TRAF6 has recently been implicated in the IL-1 signaling pathway and shown to complex with IRAK (Cao, Z., *et al.*, *Nature 383:*443-6 (1996)). It was therefore determined if IRAK-2 interacted with TRAF6 when coexpressed in 293T cells. Both IRAK and IRAK-2 coprecipitated with TRAF6 but not with the related TRAF2. A dominant negative version of TRAF6 [TRAF6 (298-522)] which inhibits IL-l-induced NF-kB activity, also bound both IRAK and IRAK-2. Further, IRAK-2-induced NF-kB activity was specifically inhibited by dominant negative TRAF6 (298-522) but not by a dominant negative version of TRAF2 [TRAF2 (87-501)] (Figure 5). These data are in keeping with TRAF6 acting downstream of IRAK-2, in the IL-1 mediated NF-kB signaling pathway.

Additional putative proximally participating adapters/regulators were sought by systematically looking for proteins showing homology to either Tube or IL-1RAcP. BLAST searches of the public data base revealed the cytoplasmic

domain of the IL-1RAcP to possess significant homology to MyD88 (Lord, K., *et al., Oncogene 5:*1095 (1990)). Sequence similarity between MyD88, IL-1RI and Toll has previously been reported, but the functional significance of this homology has been obscure. Interestingly, the MyD88 polypeptide has a modular structure composed of two fused module types: a N-terminal "interaction domain" (or DD for Death Domain that was initially defined in proteins involved in programmed cell death), (Feinstein, E., *et al., Trends Biochem. Sci. 20:*342-4 (1995); and Hofmann, K. & Tschopp, J., *et al., FEBS Letters 371:*321 (1995)) and a C-terminal domain related to the cytoplasmic region of IL-1RAcP, IL-1RI, Toll and the recently identified human Toll homologue (Hardiman, G., *et al., Oncogene 13:*2467-75 (1996); Hultmark, D., *Biochem. Biophys. Res. Commun. 199:*144 (1994); Bonnert, T., *et al., FEBS lett. 402:*81-84 (1997); and Medzhitov, R., *et al., Nature 388:*394 (1997)). Given the presence of these two distinct domains it was hypothesized that MyD88 might simultaneously connect a transmembrane receptor belonging to the IL-1R family with a downstream signaling mediator. To test this, the role of human MyD88 was functionally characterized.

Ectopic expression of MyD88 in 293 cells strongly induced NF-kB activity in a dose dependent manner. Similarly, a truncated version of MyD88 encoding the N-terminal domain (DD), MyD88 (1-151), activated NF-kB albeit to a lesser extent. In contrast, the C-terminal region, MyD88 (152-296) did not induce any luciferase activity (Figure 6A). Significantly, MyD88-induced NF-kB activity was specifically inhibited by TRAF6 but not TRAF2 dominant negative expression constructs suggesting that TRAF6 and MyD88 likely participate in the same signaling pathway and that TRAF6 functions downstream of MyD88 (Figure 6B). It was next tested whether MyD88 (152-296) could act as a dominant negative inhibitor of IL-1Rs-induced NF-kB activity; MyD88 (152-296) specifically inhibited IL-1Rs-induced but not TNFR2-induced NF-kB activation. A dominant negative version of TRAF6 [TRAF6 (289-522)] similarly inhibited IL-1Rs-induced but not TNFR2-induced NF-kB activation; in contrast, a dominant negative

version of TRAF2 [TRAF2 (87-501)] abrogated TNFR2-induced, but not IL-1Rs-induced, NF-kB activity confirming the specificity of effects observed with MyD88 (152-296).

Given the significant sequence homology existing between MyD88 and the IL-1RAcP, it was investigated whether the two could interact. Upon coexpression in 293T cells, MyD88 and IL-1RAcP formed an immunoprecipitable complex. IL-1RI, which shows weaker sequence similarity to MyD88, did not associate with MyD88 under these experimental conditions. Domain mapping studies revealed that the sequence homologous C-terminal region of MyD88 was sufficient for binding to the IL-1RAcP cytoplasmic domain (Figures 7A-7B) consistent with a hemophilic interaction.

In an effort to molecularly order the proximal components of the IL-1R signaling complex identified herein, it was tested whether the dominant negative mutant versions of MyD88 and IRAK-2 could inhibit the active forms of the others. A dominant negative version of MyD88 completely abrogated IL-1Rs-induced NF-kB activation but failed to inhibit IRAK-2-induced NF-kB activation (Figures 8A-8C). On the other hand, dominant negative versions of IRAK-2, significantly inhibited both IL-1Rs- and MyD88-induced NF-kB activity. These results are consistent with MyD88 acting upstream of IRAK-2 in the IL-1R signaling pathway.

Given the presence of a N-terminal "interaction domain" (DD) in both MyD88 and IRAK-2 (Feinstein, E., *et al.*, *supra*; and Hofmann, K. & Tschopp, J., *supra*)) it was tested whether these two proteins could interact. It was found that MyD88 specifically coprecipitated with IRAK-2. Significantly a truncated version of IRAK-2 lacking the N-terminal domain (DD) [IRAK-2 (97-590)], that failed to induce NF-kB activation, also failed to associate with MyD88; similarly, the version of MyD88 (152-296) that was unable to induce NF-kB activity, was also impaired in its ability to bind IRAK-2 lending functional credence to this interaction.

Taken together these results support a model wherein MyD88 acts as an adapter/regulator in the IL-1R signaling complex by independently interacting with IL-1RAcP and IRAK-2. However, we were unable, under these experimental conditions, to assemble a multimolecular complex between MyD88, IRAK-2 and the IL-1Rs. This is consistent with the possibility that MyD88 is only transiently recruited to the IL-1R signaling complex where it subsequently regulates IRAK-2's activity.

Methods

cDNA cloning and analysis.

A partial cDNA clone was used to screen a human HUVEC cDNA library. Hybridizing clones were characterized by automated DNA sequencing. Alternatively the sequence corresponding to aa, 391 to 570 of IL-1RAcP was used to search the NCBI Gene Bank nr database. Human and murine MyD88 cDNAs were identified as having statistically significant homology to IL-1RAcP. Sequence assembly, comparison and alignment were performed using DNASTAR software.

Expression vectors.

Mammalian expression vectors encoding Flag-TRAF6, Flag-TRAF6 (289-522), Flag-TRAF2, Flag-TRAF2 (87-501), NIK (KK429-430AA), ELAM-Luciferase reporter plasmid, Flag-IL-1RAcP and IRAK have been previously described ((Cao, Z., *et al., Nature 383:*443-6 (1996); Chinnaiyan, A., *et al., Science 274:*990-92 (1996); Malinin, N.L., *et al., Nature 385:5:*540-4 (1997); and Rothe, M., *et al., Science 269:*1424-7 (1995)). AU1-IRAK-2 (1-96), AUl-MyDS88, AU-l-MyD88 (152-296) and HA-MyD88 (1-151) were PCR amplified from a HUVEC cDNA library using custom-made oligonucleotide primers encoding the AU1 or HA epitope tag. Amplified fragments were cloned into the mammalian expression vector pCDNA3 (Invitrogen). IRAK-2-MyC and IRAK-2 (97-590)-MyC were obtained hy PCR amplification and cloned in frame into

pCDNA3-MyC-His vector (Invitrogen). Flag-IL-1RI and Flag-ΔIL-1RI were similarly obtained by PCR amplification from the HUVEC cDNA library and sub cloned in frame into pCMV-1-Flag expression vector.

Transfection and coimmunoprecipitation.

Human embryonic 293 or 293T cells were transiently transfected by calcium phosphate method with the indicated plasmids. The total amount of DNA was kept constant. 24-36 hours after transfection, cells were lysed in 0.5 ml buffer (1% NP40, 150 mM NaCl, 50 mM Tris, 1 mM EDTA and protease inhibitors cocktail). Cell lysates were adjusted to 0.7 M NaCl and the indicated antibodies were added for 1 to 4 hours. Immune complexes were precipitated by the addition of protein-G-Sepharose (Sigma). After extensive washing, the Sepharose heads were boiled in sample buffer and the eluted proteins fractionated by SDS-PAGE. Subsequent protein immunoblotting was performed as described (Chinnaiyan, A., *et al., Cell 81:*505-12 (1995)).

NF-kB luciferase assay.

Cells were transfected with 0.1 μg ELAM-Luciferase reporter plasmid, 0.2 μg pCMV-βGal and the indicated expression vectors; total amount of transfected DNA was kept constant by supplementation with empty vector. Relative NF-kB activity was calculated by normalizing relative luciferase activity with βGal activity as previously described (Cao, Z. *et al., Nature 383:*443-446 (1996).

Example 2: Tissue distribution of IRAK-2 mRNA expression

Northern blot analysis is carried out to examine IRAK-2 gene expression in human tissues, using methods described by, among others, Sambrook *et al.*, cited above. A cDNA probe containing the nucleotide sequence corresponding to the open reading frame of the IRAK-2α protein (SEQ ID NO:1) is labeled with ^{32}P using the *redi*prime™ DNA labeling system (Amersham Life Science),

according to manufacturer's instructions. After labeling, the probe is purified using a CHROMA SPIN- 100™ column (Clontech Laboratories, Inc.), according to manufacturer's protocol number PT1200-1. The purified labeled probe is then used to examine various human tissues for IRAK-2 mRNA.

Multiple Tissue Northern (MTN) blots containing various human tissues (H) are obtained from Clontech and examined with the labeled probe using ExpressHyb™ hybridization solution (Clontech) according to manufacturer's protocol number PT1190-1. Following hybridization and washing, the blots are mounted and exposed to film at -70°C overnight, and films developed according to standard procedures.

It will be clear that the invention may be practiced otherwise than as particularly described in the foregoing description and examples.

Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, are within the scope of the appended claims.

The entire disclosure of all publications (including patents, patent applications, journal articles, laboratory manuals, books, or other documents) cited herein are hereby incorporated by reference.

What Is Claimed Is:

1. An isolated nucleic acid molecule comprising a polynucleotide having a nucleotide sequence at least 95% identical to a sequence selected from the group consisting of:

(a) a nucleotide sequence encoding a polypeptide comprising amino acids from about 1 to about 590 in SEQ ID NO:2;

(b) a nucleotide sequence encoding a polypeptide comprising amino acids from about 2 to about 590 in SEQ ID NO:2;

(c) a nucleotide sequence encoding a polypeptide comprising amino acids from about 1 to about 625 in SEQ ID NO:4;

(d) a nucleotide sequence encoding a polypeptide comprising amino acids from about 2 to about 625 in SEQ ID NO:4;

(e) a nucleotide sequence encoding a polypeptide having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 209340; and

(f) a nucleotide sequence complementary to any of the nucleotide sequences in (a), (b), (c), (d), or (e).

2. An isolated nucleic acid molecule comprising a polynucleotide which hybridizes under stringent hybridization conditions to a polynucleotide having a nucleotide sequence identical to a nucleotide sequence in (a), (b), (c), (d), (e), or (f) of claim 1 wherein said polynucleotide which hybridizes does not hybridize under stringent hybridization conditions to a polynucleotide having a nucleotide sequence consisting of only A residues or of only T residues.

3. An isolated nucleic acid molecule comprising a polynucleotide which encodes the amino acid sequence of an epitope-bearing portion of an IRAK-2 polypeptide having an amino acid sequence in (a), (b), (c), (d), or (e) of claim 1.

4. The isolated nucleic acid molecule of claim 3, which encodes an epitope-bearing portion of an IRAK-2 polypeptide selected from the group consisting of: a polypeptide comprising amino acid residues from about 96 to about 193 in SEQ ID NO:2 or SEQ ID NO:4; a polypeptide comprising amino acid residues from about 207 to about 254 in SEQ ID NO:2 OR SEQ ID NO:4; a polypeptide comprising amino acid residues from about 293 to about 316 in SEQ ID NO:2 or SEQ ID NO: 4; a polypeptide comprising amino acid residues from about 416 to about 472 in SEQ ID NO:2 or SEQ ID NO:4; a polypeptide comprising amino acid residues from about 487 to about 541 in SEQ ID NO:2 or SEQ ID NO:4; and a polypeptide comprising amino acid residues from about 559 to about 619 in SEQ ID NO:4.

5. An isolated nucleic acid molecule, comprising a polynucleotide having a sequence selected from the group consisting of:

(a) the nucleotide sequence of a fragment of the sequences shown in SEQ ID NO:1 or SEQ ID NO:3, wherein said fragment comprises at least 50 contiguous nucleotides of SEQ ID NO:1 or SEQ ID NO:3, provided that said isolated nucleic acid molecule is not SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, or SEQ ID NO:14, or any subfragment thereof; and

(b) a nucleotide sequence complementary to a nucleotide sequence in (a).

6. A method for making a recombinant vector comprising inserting an isolated nucleic acid molecule of claim 1 into a vector.

7. A recombinant vector produced by the method of claim 6.

8. A method of making a recombinant host cell comprising introducing the recombinant vector of claim 7 into a host cell.

9. A recombinant host cell produced by the method of claim 8.

10. A recombinant method for producing an IRAK-2 polypeptide, comprising culturing the recombinant host cell of claim 9 under conditions such that said polypeptide is expressed and recovering said polypeptide.

11. An isolated IRAK-2 polypeptide having an amino acid sequence at least 95% identical to a sequence selected from the group consisting of:

(a) amino acids from about 1 to about 590 in SEQ ID NO:2;

(b) amino acids from about 2 to about 590 in SEQ ID NO:2;

(c) amino acids from about 1 to about 625 in SEQ ID NO:4;

(d) amino acids from about 2 to about 625 in SEQ ID NO:4;

(e) the amino acid sequence of the IRAK-2 polypeptide having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 209340; and

(f) the amino acid sequence of an epitope-bearing portion of any one of the polypeptides of (a), (b), (c), (d), or (e).

12. An isolated polypeptide comprising an epitope-bearing portion of the IRAK-2 protein, wherein said portion is selected from the group consisting of: a polypeptide comprising amino acid residues from about 96 to about 193 in SEQ ID NO:2 or SEQ ID NO:4; a polypeptide comprising amino acid residues from about 207 to about 254 in SEQ ID NO:2 OR SEQ ID NO:4; a polypeptide comprising amino acid residues from about 293 to about 316 in SEQ ID NO:2 or SEQ ID NO: 4; a polypeptide comprising amino acid residues from about 416 to about 472 in SEQ ID NO:2 or SEQ 1D NO:4; a polypeptide comprising amino acid residues from about 487 to about 541 in SEQ ID NO:2 or SEQ ID NO:4; and a polypeptide comprising amino acid residues from about 559 to about 619 in SEQ ID NO:4.

13. The isolated polypeptide of claim 11, which is produced or contained in a recombinant host cell.

14. The isolated polypeptide of claim 13, wherein said recombinant host cell is mammalian.

15. An isolated nucleic acid molecule comprising a polynucleotide encoding an IRAK-2 polypeptide wherein, except for one to fifty conservative amino acid substitutions, said polypeptide has a sequence selected from the group consisting of:

(a) a nucleotide sequence encoding a polypeptide comprising amino acids from about 1 to about 590 in SEQ ID NO:2;

(b) a nucleotide sequence encoding a polypeptide comprising amino acids from about 2 to about 590 in SEQ ID NO:2;

(c) a nucleotide sequence encoding a polypeptide comprising amino acids from about 1 to about 625 in SEQ ID NO:4;

(d) a nucleotide sequence encoding a polypeptide comprising amino acids from about 2 to about 625 in SEQ ID NO:4;

(e) a nucleotide sequence encoding a polypeptide having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 209340; and

(f) a nucleotide sequence complementary to any of the nucleotide sequences in (a), (b), (c), (d), or (e).

16. An isolated IRAK-2 polypeptide wherein, except for one to fifty conservative amino acid substitutions, said polypeptide has a sequence selected from the group consisting of:

(a) amino acids from about 1 to about 590 in SEQ ID NO:2;

(b) amino acids from about 2 to about 590 in SEQ ID NO:2;

(c) amino acids from about 1 to about 625 in SEQ ID NO:4;

(d) amino acids from about 2 to about 625 in SEQ ID NO:4;

(e) the amino acid sequence of the IRAK-2 polypeptide having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 209340; and

(f) the amino acid sequence of an epitope-bearing portion of any one of the polypeptides of (a), (b), (c), (d), or (e).

17. A method for determining if a compound of interest is an agonist or antagonist of the IRAK-2 pathway, comprising:

(a) transfecting cells which express IRAK-2 with a construct comprising a reporter gene operably linked to a promoter which is activated by NF-kB;

(b) contacting said cells with a compound of interest;

(c) assaying said reporter gene expression; and

(d) comparing said reporter gene expression to a standard reporter gene expression, said standard being assayed when no contact is made with said compound of interest; whereby,

enhanced reporter gene expression over said standard indicates that said compound of interest is an agonist of the IRAK-2 pathway, and diminished reporter gene expression under said standard indicates that said compound of interest is an antagonist of the IRAK-2 pathway.

18. A method for treating an individual in need of an increased level of IRAK-2 activity comprising administering to said individual a composition comprising an isolated polypeptide of claim 11.

19. A method useful during diagnosis of a disorder, comprising:

(a) measuring IRAK-2 gene expression level in cells or body fluid of an individual; and

(b) comparing the IRAK-2 gene expression level of said individual with a standard IRAK-2 gene expression level, whereby an increase or decrease in the IRAK-2 gene expression level over said standard is indicative of an IRAK-2-related disorder.

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                10          20          30          40          50          60
1 GCA GGC GCG CCG GAG CCG GCC CCG TAG CGT GCC ATG GCC TGC TAC ATC TAC CAG CTG CCC  60
1                                                 M   A   C   Y   I   Y   Q   L   P   9

                70          80          90          100         110         120
61 TCC TGG GTG CTG GAC GAC CTG TGC CGC AAC ATG GAC GCG CTC AGC GAG TGG GAC TGG ATG  120
10  S   W   V   L   D   D   L   C   R   N   M   D   A   L   S   E   W   D   W   M   29

               130         140         150         160         170         180
121 GAG TTC GCC TCC TAC GTG ATC ACA GAC CTG ACC CAG CTG CGG AAG ATC AAG TCC ATG GAG  180
30  E   F   A   S   Y   V   I   T   D   L   T   Q   L   R   K   I   K   S   M   E   49

               190         200         210         220         230         240
181 CGG GTG CAG GGT GTG AGC ATC ACG CGG GAG CTG CTG TGG TGG TGG GGC ATG CGG CAG GCC  240
50  R   V   Q   G   V   S   I   T   R   E   L   L   W   W   W   G   M   R   Q   A   69

               250         260         270         280         290         300
241 ACC GTC CAG CAA CTT GTG GAC CTC CTG TGC CGC CTG GAG CTC TAC CGG GCT GCC CAG ATC  300
70  T   V   Q   Q   L   V   D   L   L   C   R   L   E   L   Y   R   A   A   Q   I   89

               310         320         330         340         350         360
301 ATC CTG AAC TGG AAA CCG GCT CCT GAA ATC AGG TGT CCC ATT CCA GCC TTC CCT GAC TCT  360
90  I   L   N   W   K   P   A   P   E   I   R   C   P   I   P   A   F   P   D   S   109

               370         380         390         400         410         420
361 GTG AAG CCA GAA AAG CCT TTG GCA GCT TCT GTA AGA AAG GCT GAG GAT GAA CAG GAA GAG  420
110 V   K   P   E   K   P   L   A   A   S   V   R   K   A   E   D   E   Q   E   E   129

               430         440         450         460         470         480
421 GGG CAG CCT GTG AGG ATG GCC ACC TTT CCA GGC CCA GGG TCC TCT CCA GCC AGA GCC CAC  480
130 G   Q   P   V   R   M   A   T   F   P   G   P   G   S   S   P   A   R   A   H   149

               490         500         510         520         530         540
481 CAG CCG GCC TTT CTC CAG CCT CCT GAA GAA GAT GCC CCT CAT TCC TTG AGA AGC GAC CTC  540
150 Q   P   A   F   L   Q   P   P   E   E   D   A   P   H   S   L   R   S   D   L   169

               550         560         570         580         590         600
541 CCC ACT TCG TCT GAT TCA AAG GAC TTC AGC ACC TCC ATT CCT AAG CAG GAA AAA CTT TTG  600
170 P   T   S   S   D   S   K   D   F   S   T   S   I   P   K   Q   E   K   L   L   189

               610         620         630         640         650         660
601 AGC TTG GCT GGA GAC AGC CTT TTC TGG AGT GAG GCA GAC GTG GTC CAG GCA ACC GAT GAC  660
190 S   L   A   G   D   S   L   F   W   S   E   A   D   V   V   Q   A   T   D   D   209
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FIG. 1A

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                670         680         690         700         710         720
661 TTC AAT CAA AAC CGC AAA ATC AGC CAG GGG ACC TTT GCT GAC GTC TAC AGA GGG CAC AGG  720
210 F   N   Q   N   R   K   I   S   Q   G   T   F   A   D   V   Y   R   G   H   R  229

                730         740         750         760         770         780
721 CAC GGG AAG CCA TTC GTC TTC AAG AAG CTC AGA GAG ACA GCC TGT TCA AGT CCA GGA TCA  780
230 H   G   K   P   F   V   F   K   K   L   R   E   T   A   C   S   S   P   G   S  249

                790         800         810         820         830         840
781 ATC GAA AGA TTC TTC CAG GCA GAG TTG CAG ATT TGT CTT AGA TGC TGC CAC CCC AAT GTC  840
250 I   E   R   F   F   Q   A   E   L   Q   I   C   L   R   C   C   H   P   N   V  269

                850         860         870         880         890         900
841 TTA CCT GTG CTG GGC TTC TGT GCT GCA AGA CAG TTT CAC AGC TTC ATC TAC CCC TAC ATG  900
270 L   P   V   L   G   F   C   A   A   R   Q   F   H   S   F   I   Y   P   Y   M  289

                910         920         930         940         950         960
901 GCA AAT GGT TCC CTA CAG GAC AGA CTG CAG GGT CAG GGT GGC TCG GAA CCC CTC CCC TGG  960
290 A   N   G   S   L   Q   D   R   L   Q   G   Q   G   G   S   E   P   L   P   W  309

                970         980         990         1000        1010        1020
961 CCC CAG CGT GTC AGC ATC TGC TCA GGG CTG CTC TGT GCC GTC GAG TAC CTG CAT GGT CTG 1020
310 P   Q   R   V   S   I   C   S   G   L   L   C   A   V   E   Y   L   H   G   L  329

                1030        1040        1050        1060        1070        1080
1021 GAG ATC ATC CAC AGC AAC GTC AAG AGC TCT AAT GTC TTG CTG GAC CAA AAT CTC ACC GCC 1080
330 E   I   I   H   S   N   V   K   S   S   N   V   L   L   D   Q   N   L   T   P  349

                1090        1100        1110        1120        1130        1140
1081 AAA CTT GCT CAC CCA ATG GCT CAT CTG TGT CCT GTC AAC AAA AGG TCA AAA TAC ACC ATG 1140
350 K   L   A   H   P   M   A   H   L   C   P   V   N   K   R   S   K   Y   T   M  369

                1150        1160        1170        1180        1190        1200
1141 ATG AAG ACT CAC CTG CTC CGG ACG TCA GCC GCG TAT CTG CCA GAG GAT TTC ATC CGG GTG 1200
370 M   K   T   H   L   L   R   T   S   A   A   Y   L   P   E   D   F   I   R   V  389

                1210        1220        1230        1240        1250        1260
1201 GGG CAG CTG ACA AAG CGA GTG GAC ATC TTC AGC TGT GGA ATA GTG TTG GCC GAG GTC CTC 1260
390 G   Q   L   T   K   R   V   D   I   F   S   C   G   I   V   L   A   E   V   L  409

                1270        1280        1290        1300        1310        1320
1261 ACG GGC ATC CCT GCA ATG GAT AAC AAC CGA AGC CCG GTT TAC CTG AAG GAC TTA CTC CTC 1320
410 T   G   I   P   A   M   D   N   N   R   S   P   V   Y   L   K   D   L   L   L  429
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FIG. 1B

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                1330        1340        1350        1360        1370        1380
1321 AGT GAA ATT CCA AGC AGC ACC GCC TCG CTC TGC TCC AGG AAG ACG GGC GTG GAG AAC GTG 1380
 430 S   E   I   P   S   S   T   A   S   L   C   S   R   K   T   G   V   E   N   V  449

                1390        1400        1410        1420        1430        1440
1381 ATG GCA AAG GAG ATC TGC CAG AAG TAC CTG GAG AAG GGC GCA GGG AGG CTT CCG GAG GAC 1440
 450 M   A   K   E   I   C   Q   K   Y   L   E   K   G   A   G   R   L   P   E   D  469

                1450        1460        1470        1480        1490        1500
1441 TGC GCC GAG GCC CTG GCC ACG GCT GCC TGC CTG TGC CTG CGG AGG CGT AAC ACC AGC CTG 1500
 470 C   A   E   A   L   A   T   A   A   C   L   C   L   R   R   R   N   T   S   L  489

                1510        1520        1530        1540        1550        1560
1501 CAG GAG GTG TGT GGC TCT GTG GCT GCT GTG GAA GAG CGG CTC CGA GGT CGG GAG ACG TTG 1560
 490 Q   E   V   C   G   S   V   A   A   V   E   E   R   L   R   G   R   E   T   L  509

                1570        1580        1590        1600        1610        1620
1561 CTC CCT TGG AGT GGG CTT TCT GAG GGT ACA GGC TCT TCT TCC AAC ACC CCA GAG GAA ACA 1620
 510 L   P   W   S   G   L   S   E   G   T   G   S   S   S   N   T   P   E   E   T  529

                1630        1640        1650        1660        1670        1680
1621 GAC GAC GTT GAC AAT TCC AGC CTT GAT GCC TCC TCC TCC ATG AGT GTG GCA CCC TGG GCA 1680
 530 D   D   V   D   N   S   S   L   D   A   S   S   S   M   S   V   A   P   W   A  549

                1690        1700        1710        1720        1730        1740
1681 GGG GCT GCC ACC CCA CTT CTC CCC ACA GAG AAT GGG GAA GGA AGG CTG CGG GTC ATC GTG 1740
 550 G   A   A   T   P   L   L   P   T   E   N   G   E   G   R   L   R   V   I   V  569

                1750        1760        1770        1780        1790        1800
1741 GGA AGG GAG GCT GAC TCC TCC TCT GAG GCC TGT GTT GGC CTG GAG CCT CCC CAG GAT GTT 1800
 570 G   R   E   A   D   S   S   S   E   A   C   V   G   L   E   P   P   Q   D   V  589

1801 ACA TAA 1806
 590 T   *   590
```

FIG. 1C

```
                10          20          30          40          50          60
 1 GCA GGC GCG CCG GAG CCG GCC CCG TAG CGT GCC ATG GCC TGC TAC ATC TAC CAG CTG CGC  60
 1                                                 M   A   C   Y   I   Y   Q   L   P   9

                70          80          90          100         110         120
61 TCC TGG GTG CTG GAC GAC CTG TGC CGC AAC ATG GAC GCG CTC AGC GAG TGG GAC TGG ATG 120
10  S   W   V   L   D   D   L   C   R   N   M   D   A   L   S   E   W   D   W   M  29

               130         140         150         160         170         180
121 GAG TTC GCC TCC TAC GTG ATC ACA GAC CTG ACC CAG CTG CGG AAG ATC AAG TCC ATG GAG 180
30  E   F   A   S   Y   V   I   T   D   L   T   Q   L   R   K   I   K   S   M   E  49

               190         200         210         220         230         240
181 CGG GTG CAG GGT GTG AGC ATC ACG CGG GAG CTG CTG TGG TGG TGG GGC ATG CGG CAG GCC 240
50  R   V   Q   G   V   S   I   T   R   E   L   L   W   W   W   G   M   R   Q   A  69

               250         260         270         280         290         300
241 ACC GTC CAG CAA CTT GTG GAC CTC CTG TGC CGC CTG GAG CTC TAC CGG GCT GCC CAG ATC 300
70  T   V   Q   Q   L   V   D   L   L   C   R   L   E   L   Y   R   A   A   Q   I  89

               310         320         330         340         350         360
301 ATC CTG AAC TGG AAA CCG GCT CCT GAA ATC AGG TGT CCC ATT CCA GCC TTC CCT GAC TCT 360
90  I   L   N   W   K   P   A   P   E   I   R   C   P   I   P   A   F   P   D   S  109

               370         380         390         400         410         420
361 GTG AAG CCA GAA AAG CCT TTG GCA GCT TCT GTA AGA AAG GCT GAG GAT GAA CAG GAA GAG 420
110 V   K   P   E   K   P   L   A   A   S   V   R   K   A   E   D   E   Q   E   E  129

               430         440         450         460         470         480
421 GGG CAG CCT GTG AGG ATG GCC ACC TTT CCA GGC CCA GGG TCC TCT CCA GCC AGA GCC CAC 480
130 G   Q   P   V   R   M   A   T   F   P   G   P   G   S   S   P   A   R   A   H  149

               490         500         510         520         530         540
481 CAG CCG GCC TTT CTC CAG CCT CCT GAA GAA GAT GCC CCT CAT TCC TTG AGA AGC GAC CTC 540
150 Q   P   A   F   L   Q   P   P   E   E   D   A   P   H   S   L   R   S   D   L  169

               550         560         570         580         590         600
541 CCC ACT TCG TCT GAT TCA AAG GAC TTC AGC ACC TCC ATT CCT AAG CAG GAA AAA CTT TTG 600
170 P   T   S   S   D   S   K   D   F   S   T   S   I   P   K   Q   E   K   L   L  189

               610         620         630         640         650         660
601 AGC TTG GCT GGA GAC AGC CTT TTC TGG AGT GAG GCA GAC GTG GTC CAG GCA ACC GAT GAC 660
190 S   L   A   G   D   S   L   F   W   S   E   A   D   V   V   Q   A   T   D   D  209

               670         680         690         700         710         720
661 TTC AAT CAA AAC CGC AAA ATC AGC CAG GGG ACC TTT GCT GAC GTC TAC AGA GGG CAC AGG 720
210 F   N   Q   N   R   K   I   S   Q   G   T   F   A   D   V   Y   R   G   H   R  229

               730         740         750         760         770         780
721 CAC GGG AAG CCA TTC GTC TTC AAG AAG CTC AGA GAG ACA GCC TGT TCA AGT CCA GGA TCA 780
230 H   G   K   P   F   V   F   K   K   L   R   E   T   A   C   S   S   P   G   S  249

               790         800         810         820         830         840
781 ATC GAA AGA TTC TTC CAG GCA GAG TTG CAG ATT TGT CTT AGA TGC TGC CAC CCC AAT GTC 840
250 I   E   R   F   F   Q   A   E   L   Q   I   C   L   R   C   C   H   P   N   V  269
```

FIG. 2A

```
            850         860         870         880         890         900
841 TTA CCT GTG CTG GGC TTC TGT GCT GCA AGA CAG TTT CAC AGC TTC ATC TAC CCC TAC ATG  900
270 L   P   V   L   G   F   C   A   A   R   Q   F   H   S   F   I   Y   P   Y   M  289
            910         920         930         940         950         960
901 GCA AAT GGT TCC CTA CAG GAC AGA CTG CAG GGT CAG GGT GGC TCG GAC CCC CTC CCC TGG  960
290 A   N   G   S   L   Q   D   R   L   Q   G   Q   G   G   S   D   P   L   P   W  309
            970         980         990        1000        1010        1020
961 CCC CAG CGT GTC AGC ATC TGC TCA GGG CTG CTC TGT GCC GTC GAG TAC CTG CAT GGT CTG 1020
310 P   Q   R   V   S   I   C   S   G   L   L   C   A   V   E   Y   L   H   G   L  329
           1030        1040        1050        1060        1070        1080
1021 GAG ATC ATC CAC AGC AAC GTC AAG AGC TCT AAT GTC TTG CTG GAC CAA AAT CTC ACC CCC 1080
 330 E   I   I   H   S   N   V   K   S   S   N   V   L   L   D   Q   N   L   T   P  349
           1090        1100        1110        1120        1130        1140
1081 AAA CTT GCT CAC CCA ATG GCT CAT CTG TGT CCT GTC AAC AAA AGG TCA AAA TAC ACC ATG 1140
 350 K   L   A   H   P   M   A   H   L   C   P   V   N   K   R   S   K   Y   T   M  369
           1150        1160        1170        1180        1190        1200
1141 ATG AAG ACT CAC CTG CTC CGG ACG TCA GCC GCG TAT CTG CCA GAG GAT TTC ATC CGG GTG 1200
 370 M   K   T   H   L   L   R   T   S   A   A   Y   L   P   E   D   F   I   R   V  389
           1210        1220        1230        1240        1250        1260
1201 GGG CAG GTG ACA AAG CGA GTG GAC ATC TTC AGC TGT GGA ATA GTG TTG GCC GAG GTC CTC 1260
 390 G   Q   V   T   K   R   V   D   I   F   S   C   G   I   V   L   A   E   V   L  409
           1270        1280        1290        1300        1310        1320
1261 ACG GGC ATC CCT GCA ATG GAT AAC AAC CGA AGC CCG GTT TAC CTG AAG GAC TTA CTC CTC 1320
 410 T   G   I   P   A   M   D   N   N   R   S   P   V   Y   L   K   D   L   L   L  429
           1330        1340        1350        1360        1370        1380
1321 AGT GAA ATT CCA AGC AGC ACC GCC TCG CTC TGC TCC AGG AAG ACG GGC GTG GAG AAC GTG 1380
 430 S   E   I   P   S   S   T   A   S   L   C   S   R   K   T   G   V   E   N   V  449
           1390        1400        1410        1420        1430        1440
1381 ATG GCA AAG GAG ATC TGC CAG AAG TAC CTG GAG AAG GGC GCA GGG AGG CTT CCG GAG GAC 1440
 450 M   A   K   E   I   C   Q   K   Y   L   E   K   G   A   G   R   L   P   E   D  469
           1450        1460        1470        1480        1490        1500
1441 TGC GCC GAG GCC CTG GCC ACG GCT GCC TGC CTG TGC CTG CGG AGG CGT AAC ACC AGC CTG 1500
 470 C   A   E   A   L   A   T   A   A   C   L   C   L   R   R   R   N   T   S   L  489
           1510        1520        1530        1540        1550        1560
1501 CAG GAG GTG TGT GGC TCT GTG GCT GCT GTG GAA GAG CGG CTC CGA GGT CGG GAG ACG TTG 1560
 490 Q   E   V   C   G   S   V   A   A   V   E   E   R   L   R   G   R   E   T   L  509
           1570        1580        1590        1600        1610        1620
1561 CTC CCT TGG AGT GGG CTT TCT GAG GGT ACA GGC TCT TCT TCC AAC ACC CCA GAG GAA ACA 1620
 510 L   P   W   S   G   L   S   E   G   T   G   S   S   S   N   T   P   E   E   T  529
           1630        1640        1650        1660        1670        1680
1621 GAC GAC GTT GAC AAT TCC AGC CTT GAT GCC TCC TCC TCC ATG AGT GTG GCA CCC TGG GCA 1680
 530 D   D   V   D   N   S   S   L   D   A   S   S   S   M   S   V   A   P   W   A  549
```

FIG. 2B

```
                1690        1700        1710        1720        1730        1740
1681 GGG GCT GCC ACC CCA CTT CTC CCC ACA GAG AAT GGG GAA GGA AGG CTG CGG GTC ATC GTG 1740
 550  G   A   A   T   P   L   L   P   T   E   N   G   E   G   R   L   R   V   I   V  569
                1750        1760        1770        1780        1790        1800
1741 GGA AGG GAG GCT GAC TCC TCC TCT GAG GCC TGT GTT GGC CTG GAG CCT CCC CAG GAT GTT 1800
 570  G   R   E   A   D   S   S   S   E   A   C   V   G   L   E   P   P   Q   D   V  589
                1810        1820        1830        1840        1850        1860
1801 ACA GAA ACT TCG TGG CAA ATT GAG ATC AAT GAG GCC AAA AGG AAA CTG ATG GAG AAT ATT 1860
 590  T   E   T   S   W   Q   I   E   I   N   E   A   K   R   K   L   M   E   N   I  609
                1870        1880        1890        1900        1910        1920
1861 CTG CTC TAC AAA GAG GAA AAA GTG GAC AGC ATT GAG CTC TTT GGC CCC TGA TGA CCG GAA 1920
 610  L   L   Y   K   E   E   K   V   D   S   I   E   L   F   G   P   *              625
                1930        1940        1950        1960        1970        1980
1921 CAC AGC TGA GGA CCC TTG TCC TCA GTT GGA AAG ATG AGC ATC AGA TCA AGA AAA AGG TCT 1980
                1990        2000        2010        2020        2030        2040
1981 GAG GCA GAA TCC AAG ATC TGC CAG GAA ACA CAC AAC AAA ACA TCT GCT GTC CTG GGT GGG 2040
                2050        2060        2070        2080        2090        3000
2041 AGG GAA ACT TCA TTT CAC TGG AAT GAG TTG GGA GAG AAA GGC CCT CAG CTT TTA GAG ACA 2100
                2110        2120        2130        2140        2150        2160
2101 CAA AAA TCC ATG AAG TCT CTT CCT TTC TGG GCT TTG TTA GTC AGA GCA GGG GAT CAG AGG 2160
                2170        2180        2190        2200        2210        2220
2161 AGA CTG AAG CAG AAA CCC TGC ACA CGG GCC CAG GAT GTG GCT GAT TTT GTG GTT CCG GGG 2220
                2230        2240        2250        2260        2270        2280
2221 AGT ATG TGA TGA TAA TCA CCC CCA GCA GAT TCC ATT ACC TCA GCA GCT CTT GTT CCC CGG 2280
                2290        2300        2310        2320        2330        2340
2281 CCA CTG GCA GTT CTG CAA TGC CAT AGC ATT TTC CAG AGC TAA GAT CTC TGG GTT GTA TTT 2340
                2350        2360        2370        2380        2390        2400
2341 GCT GAC AGC CTG CAA GCT TGC ATG CTC TGA AAG ATT TTT TTA GTT TTT AAT TTT TTT GTA 2400
                2410        2420        2430        2440        2450        2460
2401 AAA ATG GGG TCT CGC TTT GTT GGC GCA ATC CTC CCA CCT CAG ACT CCC AAA GTG CTG GAA 2460
                2470        2480        2490        2500        2510        2520
2461 TTA CAT TGG GAA CCA CTG TGC CTG GCC TGG AAA ACT TCC AAC TTG TGT TCT CAG TGC AGT 2520
                2530        2540        2550        2560        2570        2580
2521 TCT GAC TCA CCT CTC TGG GCC TCA GGT TCT ACA AAT GCC AGA CAC CTA GCG AAG AGC TCT 2580
                2590        2600        2610        2620        2630        2640
2581 GCA GGC TTT CCA CTG CCT GTA TTG GAA ATC TTG CAA TTC ACA TAA TTA TTC AGT CAC TGC 2640
                2650        2660        2670        2680        2690        2700
2641 CTG GTA CCT TTA TCT TCC CAT CCC ATT AAT GTT AGT GTT TTT TAA TGG AGC TTT TAT TCT 2700
                2710        2720        2730        2740        2750        2760
2701 GAG AAT ATG TGT TCG TCT GTT TGT TTG TTT TTT GAG ACA GAG TCT CAC TTT GTC ACC CAG 2760
                2770        2780        2790        2800        2810        2820
2761 GCT GGA GTG CAG TGG CAC GAT CTC AGC TCA CTG CAA GCT GTG CCT CTC AGG TTT CAA GTG 2820
```

FIG. 2C

```
            2830        2840        2850        2860        2870        2880
2821 ATT CTC CTG CCT CAG CCT CCT GAG TAG ATG GGA CTG TAG GCA CCT GCC ACT ATG CCT GGC 2880
            2890        2900        2910        2920        2930        2940
2881 TAA TTT TTG TGT TTT TAG TAG AGA CAG GGT TTC ACC ATA TTG GCC AGG CTG GTC TCG AAC 2940
            2950        2960        2970        2980        2990        3000
2941 TAC TGA CCT CGT GAT CTG CCC GCC TTG GCC TAT CAA AGT GTT GGG ATT ACA GGC TTG AGC 3000
            3010        3020        3030        3040        3050        3060
3001 CAC CGC ACC CGG CCG AGA ATA TGT GTT GTT ATT TAT GAC TGG ATT ATG AAG AAT CAG GAG 3060
            3070        3080        3090        3100        3110        3120
3061 AAT GCA TTT CAT GTC TGA TTC TGC TGC TAA TTA AGT CAA TCA TTT AAT TTT TGG GAC CTC 3120
            3130        3140        3150        3160        3170        3180
3121 AGT TTC TTT GTA AGT AAA ATA ACA CCT GCT TGT TCT TCA TCC CTG GGC TGT TGG GAG GAA 3180
            3190        3200        3210        3220        3230        3240
3181 CAG ATG AGA CAG TGG CTA TAG AAG CAC TTG GAA AAT GCA CTT GTC CTG TTT TGT AAA ATA 3240
            3250        3260        3270        3280        3290        3300
3241 AAA AGG TAT TAA ATG TGT ATT TCT GCC ATG TAC CTA ATG ATT ATT CAG TGC GTA TAT ATC 3300
            3310        3320        3330        3340        3350        3360
3301 TGA AAA GTC ATG TTG CAA ATC TTT CTG TGA AAC AGA TGC TAT TTT AAA TTC ACT GGG AGA 3360
            3370        3380        3390        3400        3410        3420
3361 AAT ATC CTA TTT AAA GTA ATC TAT AGT AAT TTC TTT TTA TAT AAT AAA AAT ATA TTT GTA 3420
            3430        3440        3450
3421 AAG TCG AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA    3459
```

FIG. 2D

```
1    MAGGPGPGEPAAPGAQ----------HFLYEVPPWVM-----CRFYKVMD   IRAK
1    MSGVQTAEAEAQAQNQANGNRTRSRSHLDNTMAIRLLPLPVRAQLCAHLD   Pelle
1    MAC-----------------------YIYQLPSWVL-----DDLCRNMD   HNFIP11X IRAK-2 Alpha
1    MAC-----------------------YIYQLPSWVL-----DDLCRNMD   HNFIP11XX IRAK-2 Beta

36   ALEPADWCQFAALIVRDQTELRLCERSGQRTASV------LWPWINR-NA   IRAK
51   ALDV--WQQLATAVKLYPDQVEQISSQKQR--GRSASNEFLNIWGGQYNH   Pelle
22   ALSEWDWMEFASYVITDLTQLRKI-KSMERVQGVSITRELLWWWGMR-QA   HNFIP11X IRAK-2 Alpha
22   ALSEWDWMEFASYVITDLTQLRKI-KSMERVQGVSITRELLWWWGMR-QA   HNFIP11XX IRAK-2 Beta

79   RVADLVHILTHLQLLRARDIITAWHPPAPLPSPGTTAPRPSSIPAPAEAE   IRAK
97   TVQTLFALFKKLKLHNAMRLIKDYVSED--------LHKYIPRSVPTISE   Pelle
70   TVQQLVDLLCRLELYRAAQIILNWKPAPEIRCPIPAFPDSVKPEKPLAAS   HNFIP11X IRAK-2 Alpha
70   TVQQLVDLLCRLELYRAAQIILNWKPAPEIRCPIPAFPDSVKPEKPLAAS   HNFIP11X IRAK-2 Beta

129  AWSPRKLPSSASTFLSPAFPGSQTHSGPELG---LVPS----PASLWPPP   IRAK
139  LRAAPD--SSAKVNNGPPFPSSSGVSNSNNNRTSTTATEEIPSLE-----   Pelle
120  VRKAEDEQEEGQPVRMATFPGPGSSPARAHQPAFLQPPEEDAPHSLRSDL   HNFIP11X IRAK-2 Alpha
120  VRKAEDEQEEGQPVRMATFPGPGSSPARAHQPAFLQPPEEDAPHSLRSDL   HNFIP11X IRAK-2 Beta

172  PSPAPSSTKPGPESSVSLLQGARPSPFCWPLCEISRGTHNFSEELKIGEG   IRAK
182  --SLGNIHISTVQRAAESLLEID--------YAELENATDGWSPDNRLGQG   Pelle
170  PTSSDSKDFSTSIPKQEKLLSLAGDSLFWSEADVVQATDDFNQNRKISQG   HNFIP11X IRAK-2 Alpha
170  PTSSDSKDFSTSIPKQEKLLSLAGDSLFWSEADVVQATDDFNQNRKISQG   HNFIP11X IRAK-2 Beta

222  GFGCVYRAVMRNTVYAVKRLK---ENADLEWTAVKQSFLTEVEQLSRFRH   IRAK
223  GFGDVYRGKWKQLDVAIKVMNYRSPNIDQKMVELQQSYN-ELKYLNSIRH   Pelle
220  TFADVYRGHRHGKPFVFKKLR---ETACSSPGSIERFFQAELQICLRCCH   HNFIP11X IRAK-2 Alpha
220  TFADVYRGHRHGKPFVFKKLR---ETACSSPGSIERFFQAELQICLRCCH   HNFIP11X IRAK-2 Beta

269  PNIVDFAGYCAQNGFYCLVYGFLPNGSLEDRLHCQTQACP--PLSWPQRL   IRAK
272  DNILALYGYSIKGQKPCLVYQLMKGGSLEARLRAHKAQNPLPALTWQQRF   Pelle
267  PNVLPVLGFCAARQFHSFIYPYMANGSLQDRLQGQG-GSE--PLPWPQRV   HNFIP11X IRAK-2 Alpha
267  PNVLPVLGFCAARQFHSFIYPYMANGSLQDRLQGQG-GSD--PLPWPQRV   HNFIP11X IRAK-2 Beta

317  DILLGTARAIQFLHQD-SPSLIHGDIKSSNVLLDERLTPKLGDFGLARFS   IRAK
322  SISLGTARGIYFLHTARGTPLIHGDIKPANILLDQCLQPKIGDFGLVR--   Pelle
314  SICSGLLCAVEYLH---GLEIIHSNVKSSNVLLDQNLTPKLAH-PMAHLC   HNFIP11X IRAK-2 Alpha
314  SICSGLLCAVEYLH---GLEIIHSNVKSSNVLLDQNLTPKLAH-PMAHLC   HNFIP11X IRAK-2 Beta

366  RFAGSSPSQSSMVARTQTVRGTLAYLPEEYIKTGRLAVDTDTFSFGVVVL   IRAK
370  ----EGPKSLDAVVEVNKVFGTKIYLPPEFRNFRQLSTGVDVYSFGIVLL   Pelle
360  --PVNKRSKYTMM-KTHLLRTSAAYLPEDFIRVGQLTKRVDIFSCGIVLA   HNFIP11X IRAK-2 Alpha
360  --PVNKRSKYTMM-KTHLLRTSAAYLPEDFIRVGQVTKRVDIFSCGIVLA   HNFIP11X IRAK-2 Beta
```

FIG.3A

```
416 ETLAGQRAVKTHGARTKYLKDLVEEEAEEAGVALRSTQSTLQAGLAADAW   IRAK
416 EVFTG-RQVTDRVPENETKKNLLD-------------------YVKQQW   Pelle
407 EVLTGIPAMDNNRSPV-YLKDLLLSEIPSSTASLCSRKTGVENVMAKE--  HNFIP11X IRAK-2 Alpha
407 EVLTGIPAMDNNRSPV-YLKDLLLSEIPSSTASLCSRKTGVENVMAKE--  HNFIP11X IRAK-2 Beta

466 AAPIAMQIYKKHLDPRPGPCPPELGLGLGQLACCCLHRRAKRRPPMTQVY   IRAK
445 RQNR-MELLEKHLAAPMGK-------ELDM--CMC--------------   Pelle
454 -------ICQKYLEKGAGRLPEDCAEALATAACLCLRRRNTS--------  HNFIP11X IRAK-2 Alpha
454 -------ICQKYLEKGAGRLPEDCAEALATAACLCLRRRNTS--------  HNFIP11X IRAK-2 Beta

516 ERLEKLQAVVAGVPGHLEAASCIPPSPQENSYVSSTGRAHSGAAPWQPLA   IRAK
470 -----------AIEAGLH--------------------------------   Pelle
489 --LQEVCGSVAAVEERL-------------------RGRETLLPWSGLS   HNFIP11X IRAK-2 Alpha
489 --LQEVCGSVAAVEERL-------------------RGRETLLPWSGLS   HNFIP11X IRAK-2 Beta

566 APSGASAQAAEQLQRGPNQPVESDESLGGLSAALRSWHLTPSCPLDPAPL   IRAK
477 ------------------------------------------------   Pelle
517 EGTGSSSNTPEETDDVDNSSLDASSSMS-----VAPWA-GAATPLLPT--  HNFIP11X IRAK-2 Alpha
517 EGTGSSSNTPEETDDVDNSSLDASSSMS-----VAPWA-GAATPLLPT--  HNFIP11X IRAK-2 Beta

616 REAGCPQGDTAGESSWGSGPGSRPTAVEGLALGSSASSSSEPPQIIINPA   IRAK
477 ----------------------------CTALDPQDR----------PS   Pelle
559 -------ENGEGRLRVIVGREADSSSEACVGLEPPQDVT-------------  HNFIP11X IRAK-2 Alpha
559 -------ENGEGRLRVIVGREADSSSEACVGLEPPQDVTETSWQIEINEA  HNFIP11X IRAK-2 Beta

666 RQKMVQKLALYEDGALDSLQLLSSSSLPGLGLEQDRQGPEESDEFQS      IRAK4
488 MNAVLKRFEPFVTD                                       Pelle
591                                                        HNFIP11X IRAK-2 Alpha
602 KRKLMENILLYKEEKVDSIELFGP                               HNFIP11X IRAK-2 Beta
```

FIG.3B

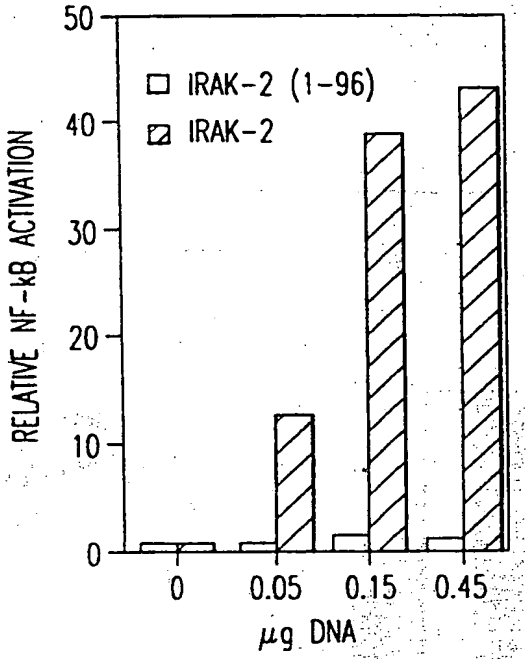

FIG. 4A

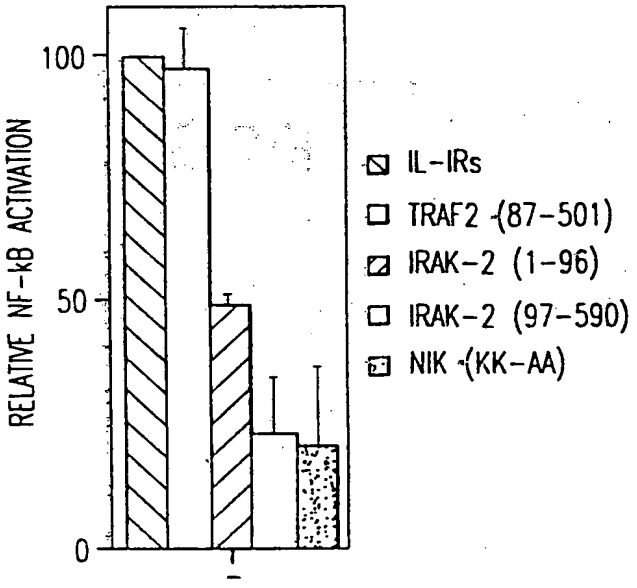

FIG. 4B

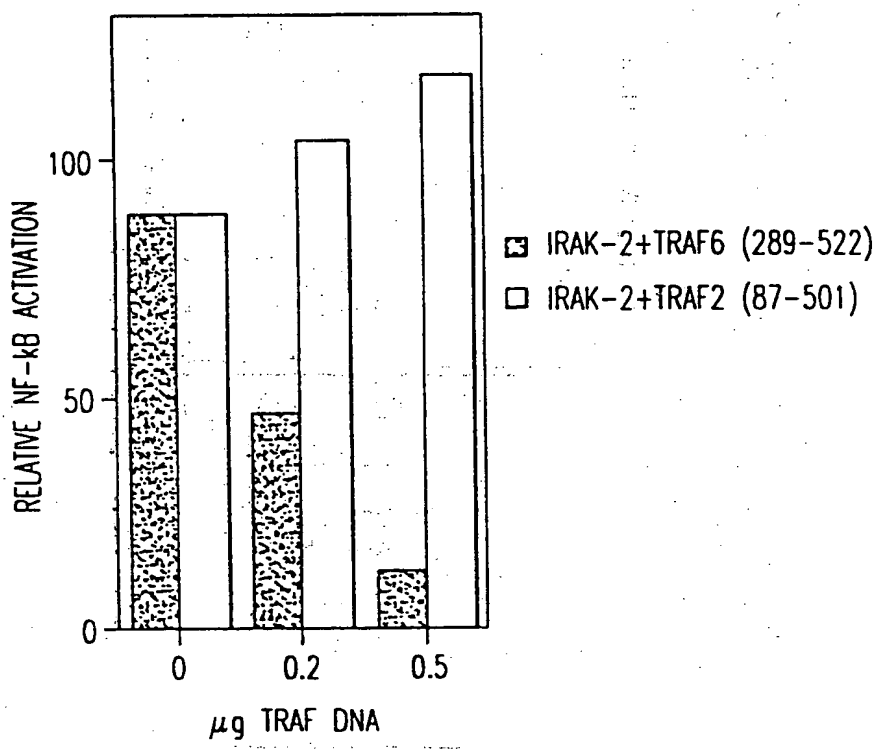

FIG.5

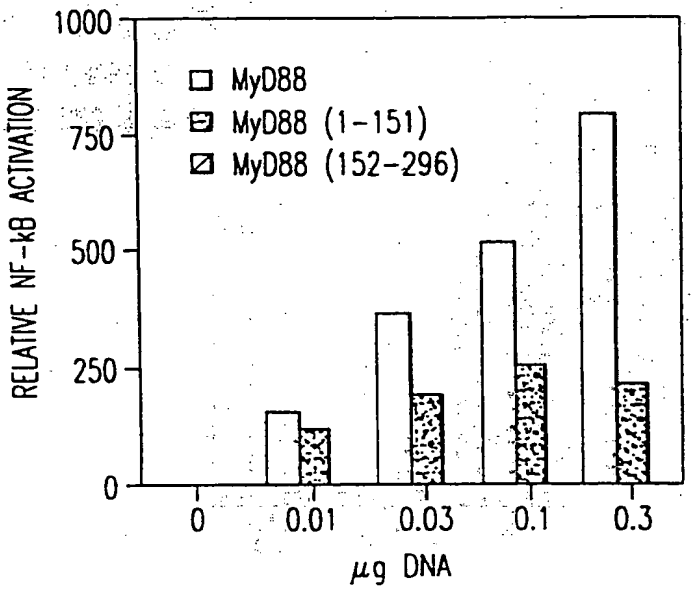

FIG. 6A

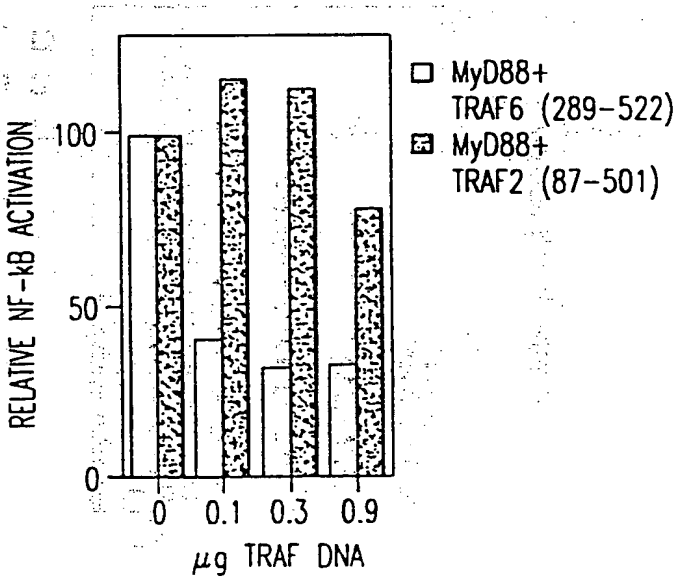

FIG. 6B

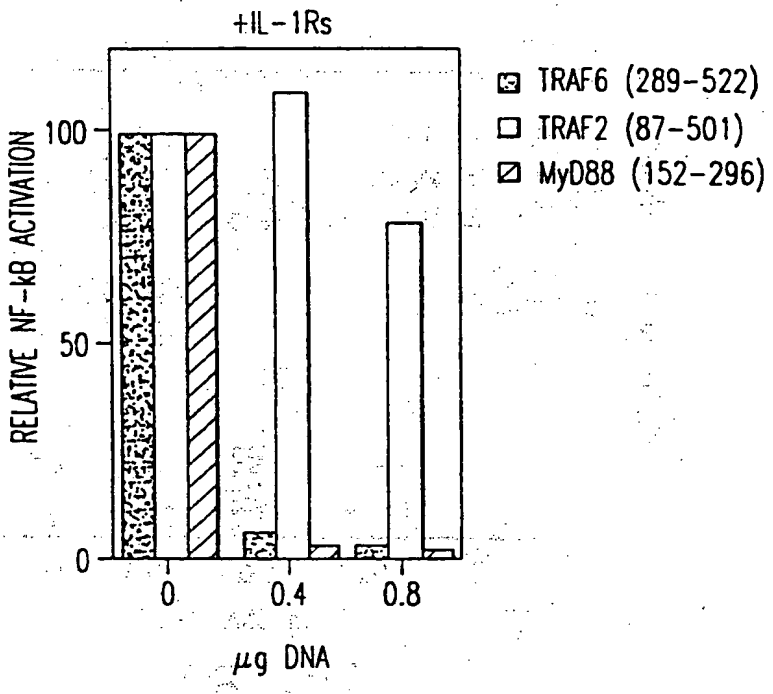

FIG. 7A

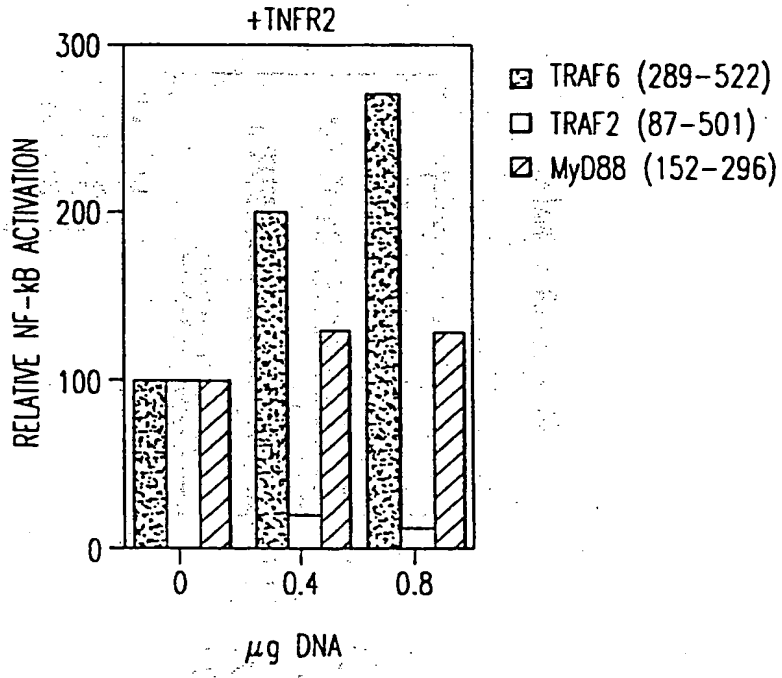

FIG. 7B

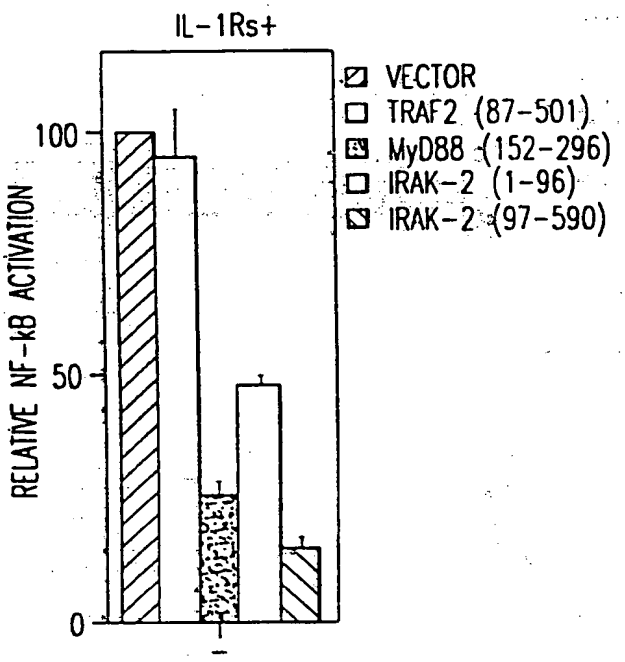

FIG. 8A

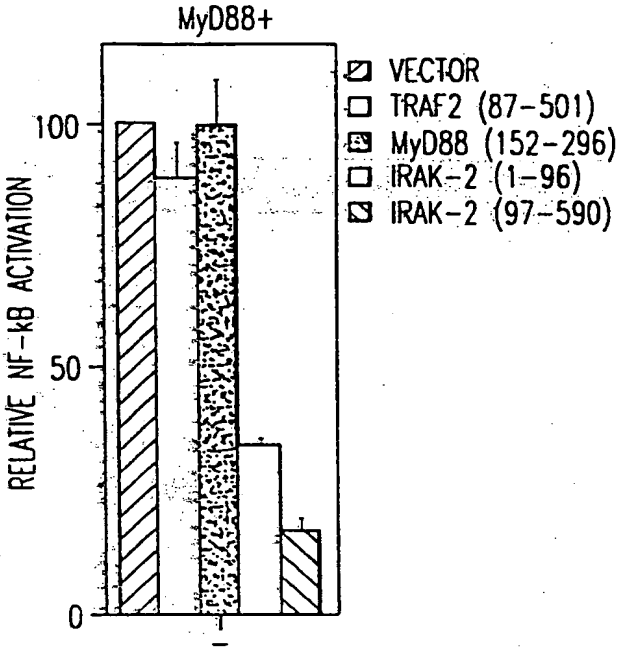

FIG. 8B

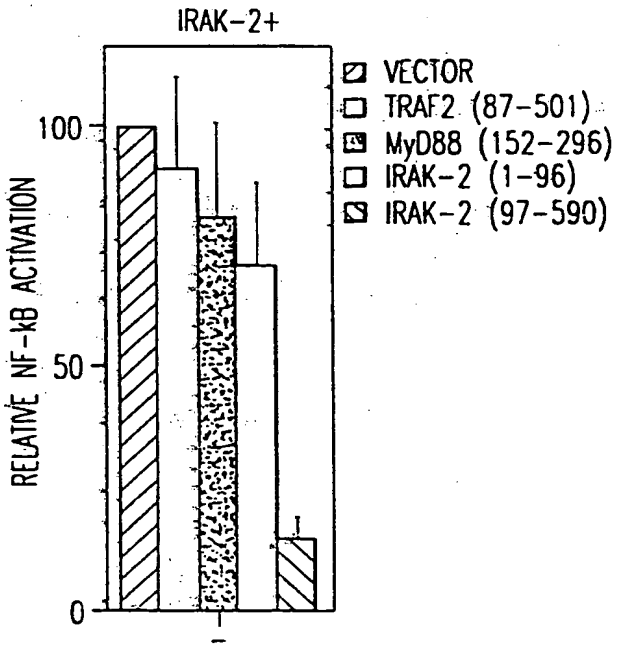

FIG. 8C

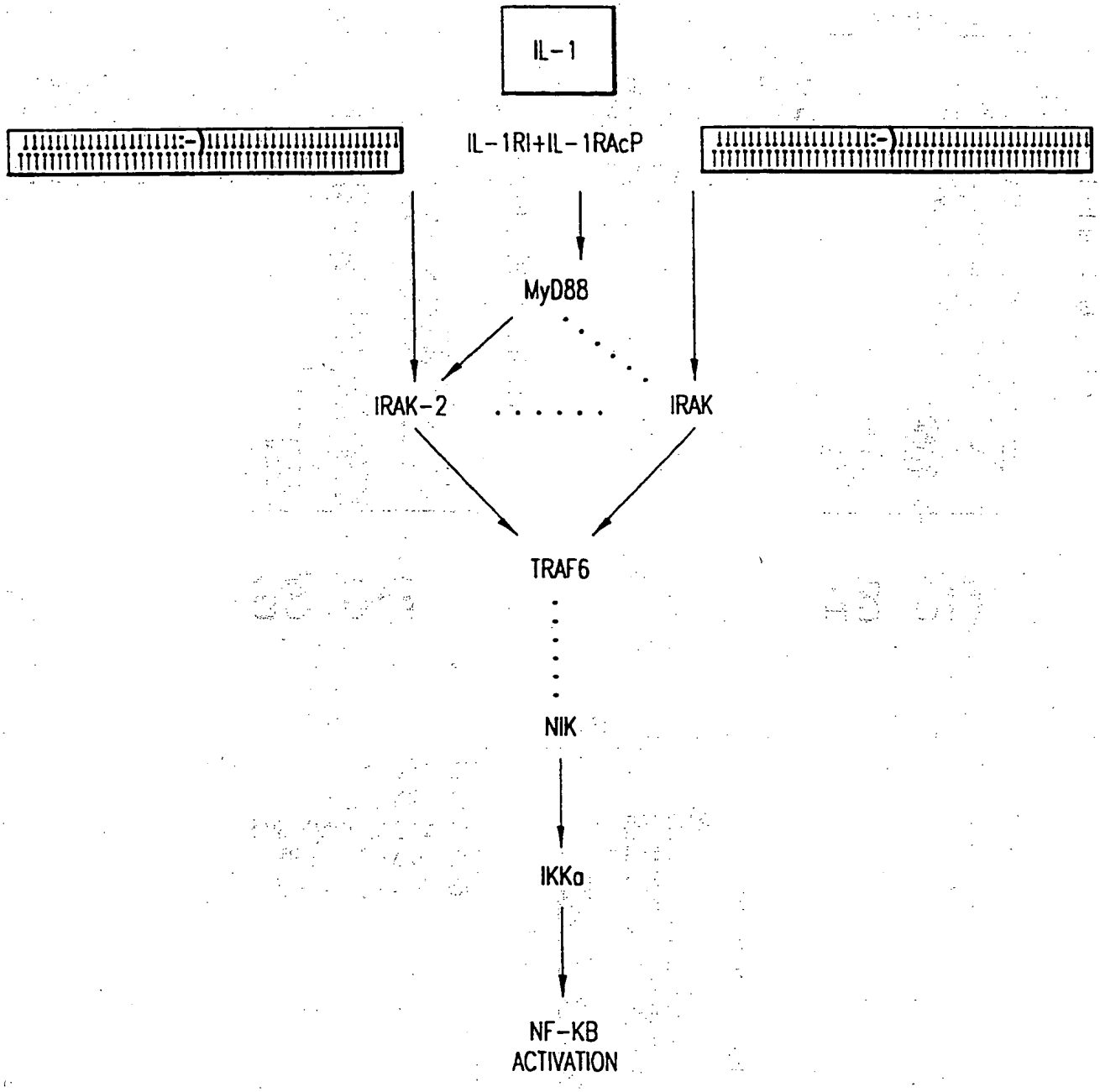

FIG.9

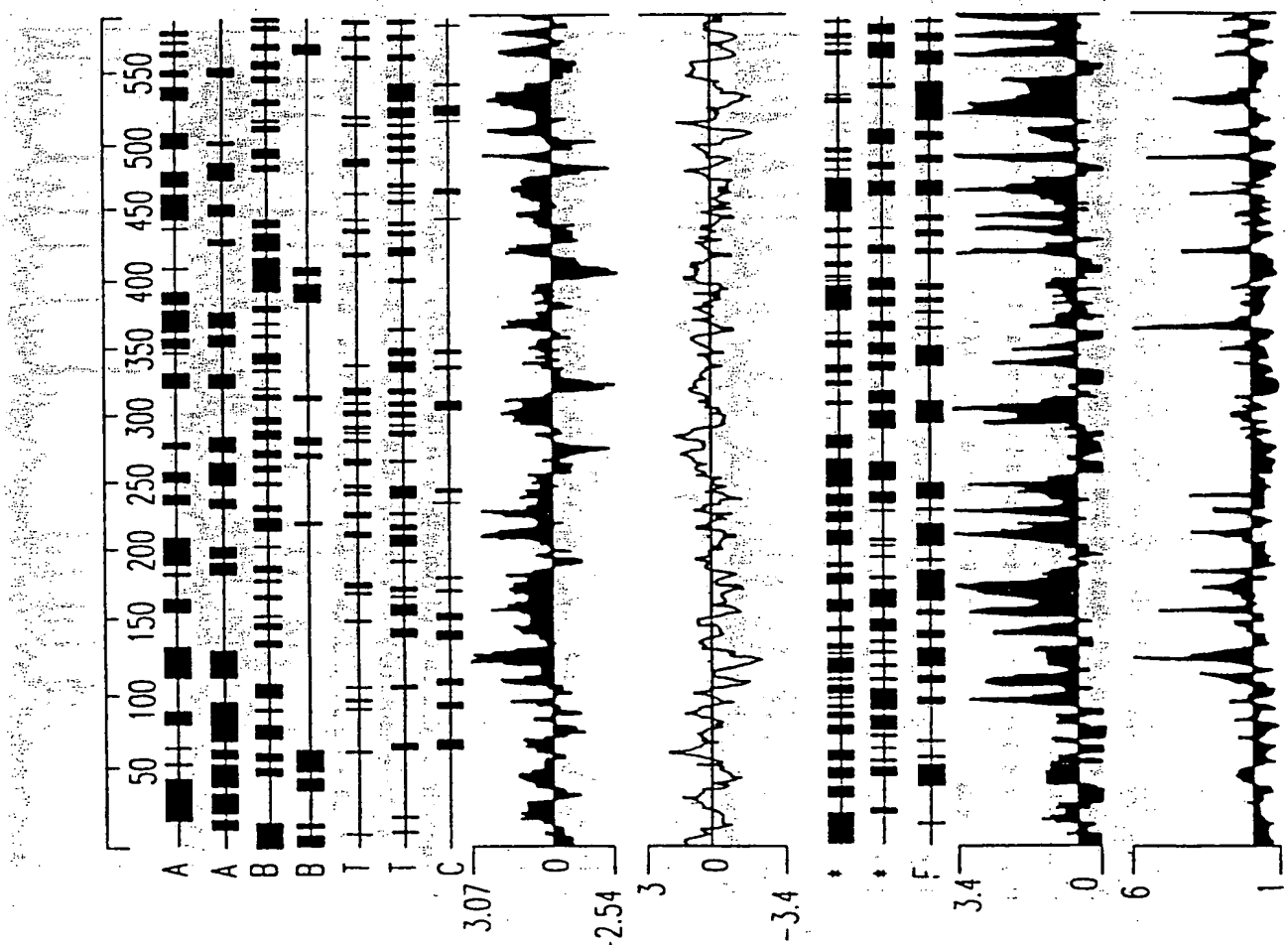

FIG.10

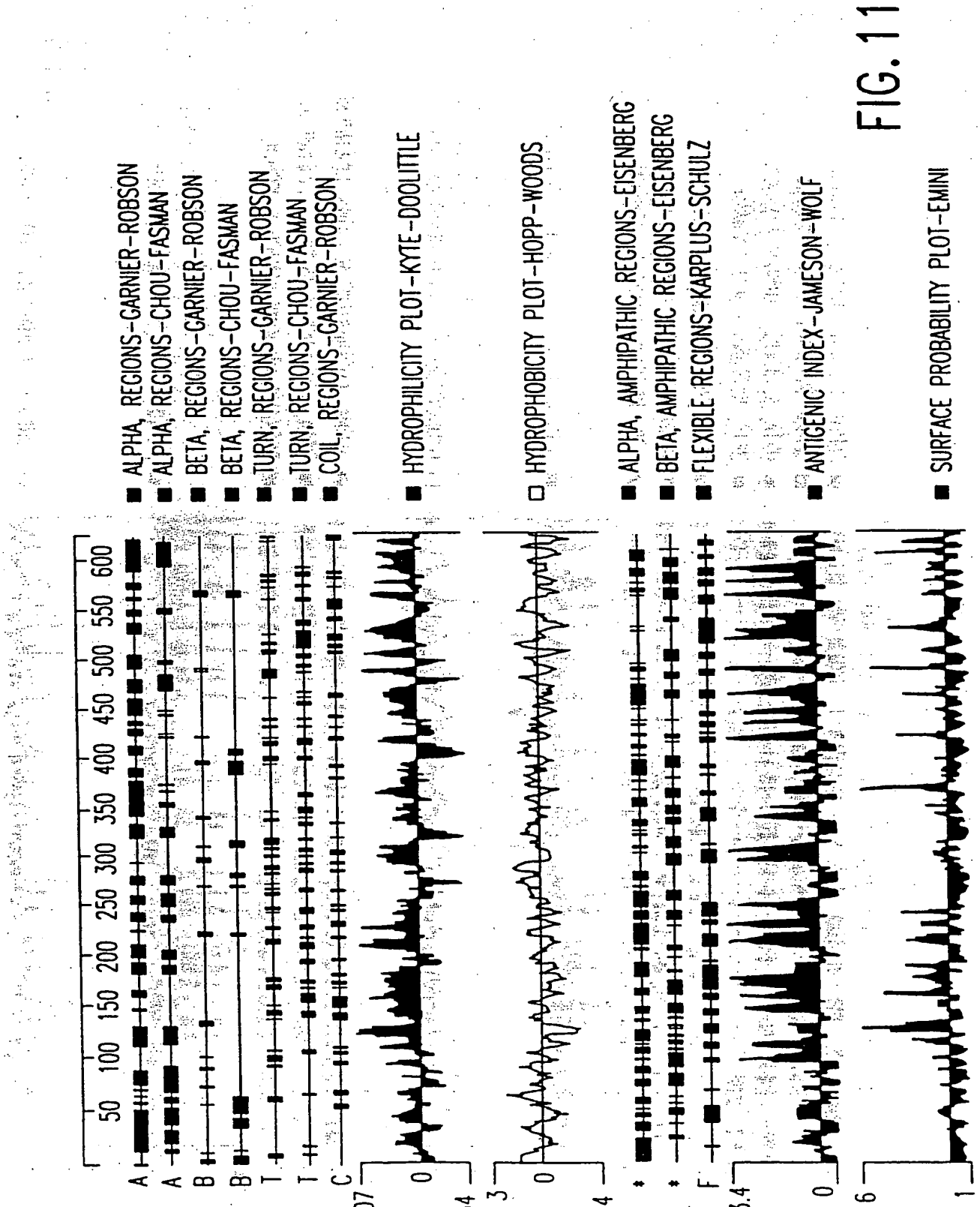

FIG. 11

INTERNATIONAL SEARCH REPORT

ernational Application No
PCT/US 98/25184

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/54 C12N9/12 G01N33/50 A61K38/45

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N G01N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	MUZIO M ET AL: "IRAK (Pelle) family member IRAK - 2 and MyD88 as proximal mediators of IL-1 signaling." SCIENCE, (1997 NOV 28) 278 (5343) 1612-5. JOURNAL CODE: UJ7. ISSN: 0036-8075., XP002099801 United States see the whole document ---	1-3, 5-11, 13-17
X	HILLIER L ET AL: "Homo sapiens cDNA clone 246238" EMEST DATABASE ENTRY HS479289, ACCESSION NUMBER N52479,18 February 1996, XP002099817 cited in the application see sequence ---	1,2
A	WO 97 00690 A (TULARIK INC) 9 January 1997 ------	

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 April 1999	03/05/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Espen, J

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 98/ 25184

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
see FURTHER INFORMATION sheet PCT/ISA/210

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Although claim 19 is directed to a diagnostic method practised on the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
Although claim 18 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 98/25184

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9700690 A	09-01-1997	AU 702844 B	04-03-1999
		AU 6176696 A	22-01-1997
		CA 2225450 A	09-01-1997
		EP 0839045 A	06-05-1998